DOCTOR OF PHILOSOPHY

Feral oilseed rape populations within a scottish landscape
Implications for GM coexistence and environmental risk assessment

Banks, Gillian

Award date:
2014

DOCTOR OF PHILOSOPHY

Feral oilseed rape populations within a scottish landscape: Implications for GM coexistence and environmental risk assessment

Gillian Banks

2014

University of Dundee

Feral Oilseed Rape Populations within a Scottish Landscape: Implications for GM coexistence and environmental risk assessment

Gillian Banks

Presented for the degree of Doctor of Philosophy
at the University of Dundee

January 2014

List of Contents

List of Tables

List of Figures

Acknowledgments

Professor Geoff Squire and Dr Cathy Hawes who provided excellent supervision and helpful advice. Thanks to Professor Stephen Hubbard and Dr Alison Karley for their valuable input at various stages of this work.

A special thanks to Mark W. Young for his patience, help and advice with GIS.

Jim McNicol (BioSS) who provided statistical help and advice.

Philip Smith for kindly proof reading this document.

Thanks to my friends and colleagues at the James Hutton Institute (Dundee).

A huge thanks to my daughter Lauren and my family for their support and efforts in keeping me sane.

Declaration

I hereby declare that the following thesis is based on the results of investigation conducted by myself, and that this thesis is my own composition. This thesis has not in whole or any part, been previously presented for a higher degree. Work other than my own is clearly stated in the text with reference to the relevant researchers of their publications.

Gillian Banks

I declare that Gillian Banks has fulfilled the conditions of the Ordinance General No.14 of Dundee and is qualified to submit the accompanying PhD thesis in application for the degree of Doctor of Philosophy.

Dr Alistair Geddes

Professor Geoffrey Squire

Abstract

In many regions of the world, the crop, oilseed rape (*Brassica napus*), is giving rise to populations of volunteer weeds in fields and feral plants outside fields, both of which can retain crop genes and hybridize with compatible wild relatives. Feral oilseed rape has received global attention as a means by which genetically modified (GM) traits may persist in the environment. There are still major uncertainties, however, over the long term environmental and economic consequences of its persistence and invasiveness, particularly in relation to GM coexistence and environmental risk assessment.

This thesis presents a demographic study of feral oilseed rape over an 11 year period from 1993 to 2004 within a 500 km^2 area of Tayside (Scotland). The number of feral oilseed rape populations increased almost five-fold during a period when the number of fields and total area cropped with oilseed rape decreased. Ferals did not usually remain at the same location for more than one or two years, and did not spread by gradual movement out from the sites of initial colonization. They persisted and spread in the region by occurring at different places each year, most likely through long range dispersal. Transport corridors hosted higher densities than farmland, in which ferals were more prevalent in areas having a high density of oilseed rape crops.

The insect communities associated with feral oilseed rape and a related ruderal plant charlock were compared to gauge the potential ecological impact of ferality. Ferals did not appear to compete with charlock but provided an additional host for

those invertebrate species already living on charlock. They also had the potential to function as a bridge for crop pests between growing seasons.

At current levels of feral oilseed rape there are unlikely to be any issues related to coexistence (i.e. ferals will bring a negligible contaminant to crops), but feral oilseed rape can persist and flower outside the range of cropped oilseed rape plants. It has become part of the native weed and wildflower community, but to date has had no major ecological impact.

The long term demographic changes in feral oilseed rape that were found in the 11 year study could not have been predicted from the initial early years when there were few populations or from prior estimates of risk carried out at small spatial scales. A long term approach is therefore needed at realistic scales for successful ecological risk assessment. The Tayside study could provide a baseline and model for assessing the ecological impact of new GM traits such as cold tolerance or insect resistance.

1. GENERAL INTRODUCTION

Feralism is the process by which crops give rise to plants that survive outside managed fields, usually in ruderal, or disturbed, habitats (Charters *et al*. 1999). These feral plants may be genetically similar to crops and able to cross pollinate with them. They also have the potential to hold genetic material that may be different from that found in crops growing nearby, as could occur if crop varieties that had specific characteristics were phased out of production. Feral plants could also potentially exchange genetic material with sexually compatible wild relatives (Elling *et al*. 2010; Lefol *et al*. 1997; Landbo *et al*. 1996).

Feralism occurs in many crops, for example in grasses (Zapiola and Mallory-Smith 2012; Zapiola *et al*. 2008), cereals (White *et al*. 2006), legumes (Bagavathiannan *et al*. 2012) and brassicas (Squire *et al*. 2011). In some instances the ferals become highly weedy and invasive such as has happened with bent grass in the US (Zapiola and Mallory-Smith 2012), making them difficult to control.

Due to their potential weediness and invasiveness, feral plants have taken on great significance in places where GM crops are being introduced, whether for cultivation (Devos *et al*. 2012; Squire *et al*. 2011; Messéan *et al*. 2009) or import for food and feed processing (Kawata 2009). It is argued that GM ferals may pose a risk to semi-natural populations and habitats (Knispel and McLachlan 2010; Bagavathiannan and Van Acker 2008) or make it difficult for GM and non-GM plants to coexist in the same landscape (Messean *et al*. 2009).

Despite the wealth of literature on feral plants, there are very few studies that have examined the spread, persistence and ecological role of ferals over time scales of more than a few years. This thesis describes and analyses a case history in which feral oilseed rape became established and spread in a region of commercial agriculture over 11 years.

To provide background and context, this Chapter summarises the history, uses and role of oilseed rape in the cropping system, the different forms of oilseed rape that exist in what is known as the *Brassica* complex, and describes the generalized life cycle of and interactions between these forms. The chapter continues by reviewing knowledge of the demography of feral oilseed rape and finally considers implications of the spread and persistence of oilseed rape for both GM coexistence and environmental risk assessment of GM crops in Europe (Messéan *et al.* 2009). The chapter ends by posing questions that are examined in the rest of the thesis.

1.1 HISTORY, USES AND FORMS OF OILSEED RAPE

Rapeseed is an ancient crop. According to Sanscrit writing, rapeseed and mustard were cultivated in India 3000 years ago. They were introduced to China and Japan about 500 to 200 BC. Greek and Roman writings from the same time also described oilseed Brassicas (Kryzmański 1998). There is evidence that the winter form of *Brassica napus* was cultivated as an oil crop in the sixteenth century in the Netherlands and from the sixteenth century until the mid-nineteenth century in England, when it almost disappeared as oilseeds and mineral oils were increasingly imported from abroad (Thirsk 1997). Its resurgence throughout the farmed landscape

in the 1970s gave rise to the questions about potential ecological impacts and coexistence that form the subject of this study.

The species of oilseed rape currently grown in the UK (*B. napus*) had been rarely noted as an arable weed before 1970. It is uncertain whether the rapeseed grown in medieval times was the oilseed form of *B. napus* or the turnip (*B. rapa*, formerly *B. campestris*). *B. napus* originated as a cross between *B. rapa* and the cabbage *B. oleracea*.

The terms rapeseed and oilseed rape are now mostly interchangeable, and the latter is used in this thesis since it much more widely used and understood in Europe than the former. Oilseed rape has a useful role as a break crop, but yields less than cereals (3.5-4.5 tonnes per hectare as opposed to 8 tonnes per hectare from some varieties of wheat). However, the yield of oilseed rape contains almost twice the energy per unit mass as cereals and generally commands a higher price at markets.

Most of the oil from this harvest is used for human consumption, particularly in food manufacture, catering and retail sales. Some of the harvested crop is used for a range of industrial purposes including lubricants, paints and surface coatings, and specific varieties of oilseed rape, known as high erucic acid rape (HEAR), are grown for some of these industrial uses. The left-over meal is predominantly used in compound animal feeds and a small market exists in certain regions for cracked whole oilseed rape for use by food manufacturers.

The EU as a whole is the largest producer of oilseed rape in the world (18 million tonnes). Production has increased markedly since the 1970s. In the UK alone, the area under oilseed rape increased from 5000 hectares in 1971 to 0.4 million hectares in 1999, an 80-fold increase in less than 30 years (Stamatelatou *et al*. 2012).

Two main factors fueled the rapid increase in agricultural land area devoted to oilseed rape cropping since the 1970s. These were the very large rises in world commodity prices for oilseeds in 1972-74 and Britain's membership of the European Economic Community (EEC) (Ward *et al*. 1985). Because of the EEC policy that the Community should become less dependent on imported vegetable oils and proteins, the price support policy helped the average market price for rapeseed to rise from around £50 per tonne in 1971-2 to over £300 in 1983 (Ward *et al*. 1985). A range of other factors contributed to the uptake of oilseed rape in the UK (Kimber, 1981):

(i) Food quality varieties of *B. napus* were bred from the original non-edible high erucic acid and high glucosinolate varieties.

(ii) Oilseed rape could be used as a break crop for cereals, interrupting the build-up of pests and diseases in cereals.

(iii) Much of the existing farm machinery, such as combine harvesters that were used for cereal crops could also be used for oilseed rape.

(iv) There was an increased demand for polyunsaturated vegetable fats which helped to lower the risk of heart disease.

1.1.1 Oilseed rape crops – trends in production

Arable regions of Scotland followed the trend in production shown elsewhere in the UK. The Scottish climate appeared to suit the crop as a number of very high yields were recorded in the 1980s (Ward *et al*. 1985).

Before 1988, government records (the June Agricultural Census) did not differentiate between winter or spring oilseed rape. Since the early 1990s, winter oilseed rape has been the preferred crop, but varied substantially in sown area between years due to weather and markets. Spring sown rape crops peaked in 1993 but subsequently were followed by a gradual decline. Figure 1.1 shows the production area in hectares of oilseed rape in Scotland from 1988 to 2007.

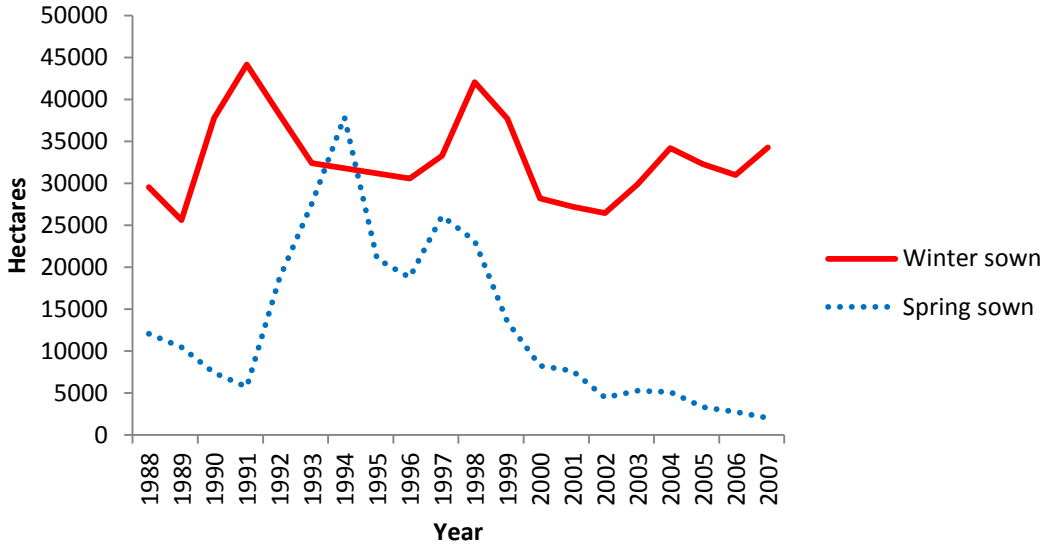


Figure 1.1 Production area of oilseed rape in Scotland 1988 – 2007, from the Abstract of Scottish Agricultural Statistics 1982 to 2007, www.scotland.gov

1.1.2 Types of oilseed rape

The characteristics of cropped oilseed rape are likely to have a strong bearing on the fitness and performance of feral populations. In the UK, winter oilseed rape is sown

in August or September, flowers between April and June, and is harvested in July or August. Spring oilseed rape is sown from March to early May, flowers from June to July and is harvested in September.

The early varieties of oilseed rape had high levels of erucic acid and glucosinolates which were found to have undesirable effects. Erucic acid produced heart lesions in laboratory animals. Accordingly, plant breeders produced “single low” varieties with low levels of erucic acid in the seeds and these started to be widely grown in the 1970s. It was also found that the glucosinolates in the seed meal broke down to give unpalatable, toxic and thyroid damaging substances, so that meal containing high concentrations could be incorporated into animal feeds in only small amounts. Plant breeders then produced “double low” varieties with low levels of glucosinolates and erucic acid in the seeds, which became widely grown in Europe during the 1980s (Kimber, 1981). Double low varieties have remained the primary types for food and feed production while high erucic rapeseed is still grown in small areas for specific industrial uses.

Within the spring and winter types, varieties of oilseed rape change rapidly, for several reasons. Firstly, breeders are able to exploit the considerable variation existing within *B. napus*, which offers great scope for selection and varietal improvement. This variation is due primarily to the outcrossing nature of the plant although, in practice, a substantial amount of self-pollination occurs (Damgaard and Kjellsson 2005). Breeders have developed quick and accurate methods of assessing quality characteristics at an early stage; tests can be undertaken on a single cotyledon or seed leaf so that, if acceptable, the rest of the seed can be grown on.

This speeds up the breeding programme and avoids multiplication of material which, in many other crops, would have to be grown for several generations to produce sufficient seed for quality tests to establish which are acceptable (Kimber 1981).

A second reason for the rapid change in varieties is the high multiplication rate of oilseed rape: a handful of breeder's seed can produce sufficient seed to sow the whole national crop within a few years, whereas it would take almost twice as long to multiply cereal seed for a similar acreage (Kimber 1981).

From the outset, the prime objective of plant breeders has been to produce higher yielding varieties and although this is still the major aim within current breeding programmes, there are also other requirements from the market which need to be realized (Table 1.1).

Table 1.1 Characteristics of new varieties required by both the market and plant breeders (Kimber 1981)

Field Characters	**Seed Characters**
Seedling vigour	High oil content
Short stem	Oil quality
Lodging resistance	Meal Quality
Early ripening	
Resistance to seed loss	
Disease resistance	
High seed yield	

The rapid turnover of varieties is particularly important as it may give weedy and feral oilseed rape the opportunity to hold traits beyond the period in which a variety is grown commercially. If ferals were to persist, then these redundant traits could also persist.

1.1.3 Agronomy, pest control and pre-harvest treatment

Winter oilseed rape is a high input crop that occurs in many types of soil and as part of several major rotations. It accompanies winter wheat and winter barley in all-winter sequences. It also (as does spring oilseed rape), acts as a break in spring cereal sequences. Winter oilseed rape typically receives more nitrogen fertilizer than winter wheat for example (e.g. 210 kg ha^{-1} as opposed to 195 kg ha^{-1}). Spring oilseed rape, in contrast, typically receives less than half the nitrogen given to winter varieties (Fertiliser Practice 2012).

Brassica crops harbour a variety of insect pests, notably cabbage stem flea beetle and aphids (virus vectors) in the autumn and pollen beetle, ceutorhynchid weevils and brassica pod midge in spring. Due to the specific chemical compounds in OSR, co-evolution with insect pests has resulted in a relatively high degree of specialism and for many OSR pests these compounds act as attractants, aiding host location. However the changed chemistry of cultivated plants has been shown to enhance the performance of some insects when compared to wild plants. Gols *et al*. (2008) showed that irrespective of insect species or the degree of dietary specialization, herbivores and parasitoids developed best on cultivated oilseed rape compared to wild populations of cabbages (*Brassica oleracea*). In the UK, more than 99% of the cropped area is now treated with chemicals. Oilseed rape on average received 3 types of herbicide, 3 fungicides, 2 insecticides and 1 molluscicide.

The main difference between oilseed rape and the cereals in relation to feralism is in the readiness of oilseed rape seed pods to shatter and drop seed. The treatment of the crop stand at and around harvest makes this problem worse. In both winter and

spring types, the crop is either cut (swathed) or sprayed with a desiccant and allowed to dry in the field before harvesting by combine. Much seed can be lost to the soil surface at this time and subsequently incorporated by ploughing. Additionally, the small seeds are readily transportable by vehicles, machinery and animals. The result has been a widespread increase in ferals and volunteer (weedy) oilseed rape not just in the fields where the crop has been grown but also more widely in the landscape, as described below in section 1.2.

1.2 THE *BRASSICA* COMPLEX – CROP, VOLUNTEER, FERAL AND WILD RELATIVE

The rise of oilseed rape since the 1970s as a crop in cereal rotations has shown that crop plants cannot be confined to the year and field in which they are sown. They give rise to weeds, usually called volunteers, as well as ferals, and all forms, depending on the species, may be able to cross pollinate with each other and with wild relatives (Elling *et al*. 2010; FitzJohn *et al*. 2007). This association of crop, volunteer, feral and wild relatives is named the *Brassica* complex in Europe.

1.2.1 Components of the complex

Crops

The crops of the *Brassica* complex comprise of oilseed, root and vegetable crops of the genus *Brassica*. Oilseed rape (*Brassica napus*) occurs as an oilseed, a forage and as a ‘root’ crop. Turnip rape (*Brassica rapa*) is a less widespread oilseed but common as a ‘root’. Cabbage (*Brassica oleracea*) occurs in a range of cultivated forms. All three species are used variously for human food and fodder and to a lesser

degree for industrial feedstocks. While *B. napus* and *B. rapa* can hybridise with each other, neither can hybridise with *B. oleracea*.

Volunteer weeds

Volunteers occur from seed dropped in fields by *B. napus*, *B. rapa* and *B. oleracea*. Of the three, *B. napus* volunteers are by far the most common and are now one of the most abundant broadleaf weeds (Debeljak *et al*. 2008), being among the top ten most abundant both in Scotland and the UK as a whole (James Hutton Institute seedbank archive, G.R.Squire personal communication). Data-mining techniques and modelling of the seedbank of arable fields (Debeljak *et al.* 2008) demonstrated that volunteer oilseed rape was more likely to be present if a previous oilseed rape crop had been grown in the same field. Volunteers also occurred in many fields in which oilseed rape had not been grown in recent times, implying origin by redistribution. It was also shown to persist for at least 8 years in arable field soils.

Volunteer rape may cause agronomic problems but can be reduced in subsequent crops by herbicides (Gruber *et al*. 2004). It is easy to control in cereal crops, but much more difficult in broad-leaved crops where broad-leaved herbicides cannot be used. The cereals and oilseeds division of the Agriculture and Horticulture Development Board (AHDB) recommends measures that farmers can use to reduce the risk of volunteer rape, including growing newer varieties with limited persistence. If different varieties are grown (food and industrial) the length of the rotations can be increased to minimize volunteer numbers and reducing the risk that the volunteers from one crop can contaminate the following crop. Shedding losses can also be minimised by harvesting when the crop is ripe (but not over-ripe). Soil

should be left uncultivated for as long as possible after harvest before sowing to reduce incorporation of seed into the seedbank and encourage germination (SCIMAC 2003). Recommendations are that soil should not be cultivated in any way after the rape harvest if the soil is dry, as this increases persistence.

Ferals

Ferals in Europe are mainly *B. napus* (Charters *et al.* 1999), but feral *B. rapa* occurs widely where the crop has been grown. Occasionally, *B. oleracea* is found by waysides and on waste land. Ferals appear closely related to current or recent crops (Pivard *et al*. 2008; Charters *et al*. 1999), suggesting they have probably arisen within the past few decades. There is no evidence that ferals have persisted from Brassica crops grown in previous centuries, for example in medieval times. Feral oilseed rape occupies mainly ruderal habitats along waysides, on soil dumps and in waste ground, but has not been found established in natural or semi-natural habitats, nor in planted forest and woodland (Pessel 2001; Crawley & Brown 1995). The feral life cycle is described in section 1.2.3.

Wild relatives

Interspecific hybridization is a common phenomenon among plants and depending on a variety of factors such as the rate of cross pollination and the fitness of hybrids, a number of outcomes are possible. If, for example, the hybrids are unviable or sterile, no further gene flow (introgression) can occur, and the species will remain genetically distinct (Chapman and Burke 2006). Alternatively, if the hybrids are viable and at least partially fertile then (1) one population may be driven to extinction (particularly if hybridization is asymmetric - e.g. from a large population

to a small population), (2) bi-lateral hybridization may result in the demise of both species and the establishment of a hybrid swarm in their place, or (3) introgression (i.e. the transfer of alleles from one taxon to another via backcrossing) may occur.

B. napus does not exist as a wild plant itself (being a hybrid between *B. rapa* and *B. oleracea*), but both its 'parents' have wild forms and it can introgress with certain other wild relatives. For introgression to occur the first requirement is that oilseed rape and a wild relative must grow within a distance over which one can transfer pollen to the other. At least a partial overlap in flowering is needed, and if insect pollinated, the sharing of common pollinators. In order for a gene or transgene to be expressed in a stable manner there also needs to be some level of genetic and structural relatedness of the genomes of both species (Devos *et al.* 2012). So, for example, *B. napus* can hybridize with *B. rapa* and *Raphanus raphanistrum* (Warwick *et al*. 2003; Jørgensen and Anderson 1994; Chévre *et al*. 1998) and wild brown mustard *B. juncea* (Di 2009); but while other "wild relatives" such as charlock (*Sinapis arvensis)* are frequently found growing in close proximity to both feral oilseed rape populations, and amongst crops of oilseed rape, hybrids are extremely rare in agricultural environments (Moyles *et al*. 2002).

Most studies have concentrated on the formation and growth of hybrids between *B. napus* and *B. rapa* (Pascher *et al*. 2010; Jørgensen 2007; Guéritaine *et al*. 2003; Warwick *et al*. 2003; Hauser *et al*. 2003). Hansen *et al*. 2003 found a high outcrossing rate between *B. napus* and *B. rapa* and offspring that were likely to survive, whereas Guéritaine *et al*. (2003) found that interspecific hybrids between *B. napus* and *R. raphanistrum* were less likely than either parent to emerge and survive

to reproduction under agronomic conditions. Wilkinson *et al*. (2003) conclude, that exposure for wild *B. rapa* growing along waterways in England is likely to be negligible with the possibility of transgene introgression being extremely small in most cases (Devos *et al*. 2012).

Summary - the Brassica complex in the UK

The most abundant forms within the *Brassica* complex in the UK are crops of winter and spring oilseed rape (*B. napus*), and volunteers and ferals descended from these crops. Flowering crops of *B. rapa* are uncommon, as are volunteer and wild forms of this species. In Scotland, no wild *B. rapa* is currently reported. The wild radish, *R. raphanistrum*, has become an uncommon weed that nevertheless occurs at high abundance in some fields.

1.2.2 The potential role of ferals in de-domestication of *Brassica* crops

Most scientists in Europe, working with the *Brassica* complex, use the term "feral" simply to mean a crop-derived plant that grows outside agricultural fields and so is no longer an integral part of the cropping cycle. By this definition, a feral growing on a roadside would still be named as such if it was genetically the same as, or similar to, a current crop. For consistency with European usage, this thesis defines feral in this way, i.e. by where the plant grows, rather than its genetic distinctiveness.

Some authors, including Gressel (2005), use the term "feral" exclusively to mean a crop plant that has reverted genetically to some previous type, which is potentially

much weedier or wild than the crop of origin. The plant has in some way reversed the process of domestication.

At the present time, the evidence that components of the *Brassica* complex are becoming detached from their crops of origin and forming populations of volunteers, ferals and hybrids with wild relatives, may indicate a first step towards de-domestication in a broad sense (rather than a genetic sense or reversion to a wild type). With present knowledge, however, it is impossible to determine whether feral populations have become persistent and self-sustaining. One of the aims of this thesis is to examine whether such populations exist.

1.2.3 Simplified feral oilseed rape lifecycle

Most forms in the *Brassica* complex, particularly in the species *B. napus* and *B. rapa*, have characteristics that confer weediness and the ability to adapt and survive (Charters *et al*. 1999). For example, asynchronous germination and flowering allow them to exploit time and weather through most of the year. Morphological plasticity allows them to flower as a very small plant and to over-winter as a mature plant and flower the next year. Of all the forms within the *Brassica complex*, ferals are likely to experience a wider range of environments and control measures than a typical crop plant or volunteer. Several factors or stages need to be considered in the lifecycle of feral oilseed in order to understand how it can successfully produce viable seeds and possibly adapt genetically. These include:

a) Dispersal of seeds from the originating crop or volunteer population.

b) The establishment of a seedbank in a ruderal environment.

c) Anthropological influences such as management of roadside verges etc.

d) Flowering followed by successful seeding and re-distribution of the feral seed.

e) Geneflow by cross-pollination both into the feral population and from the ferals to other members of the *Brassica* complex.

Each of these factors is shown at the point that they would be likely to occur in a generalized life cycle of feral oilseed rape (Figure 1.2).

(A) Dispersal of seeds from the originating oilseed rape crop or volunteer population

Seeds of oilseed rape can be dispersed on to paths and tracks or neighbouring fields by wind, birds or machinery. Pod shatter can also disperse seeds over several metres at harvest. Some of the seed falling on the soil is eaten by birds, slugs and insects, or killed by drought or frost. It has been estimated that a combine harvester can hold several kilograms of oilseed rape seeds after use (Devos 2004). Dispersal over long distances results from seeds that are spilled during transport from fields on the way to handling, storing and/or crushing facilities, or during import (Pessel *et al*. 2001; Crawley and Brown 1995).

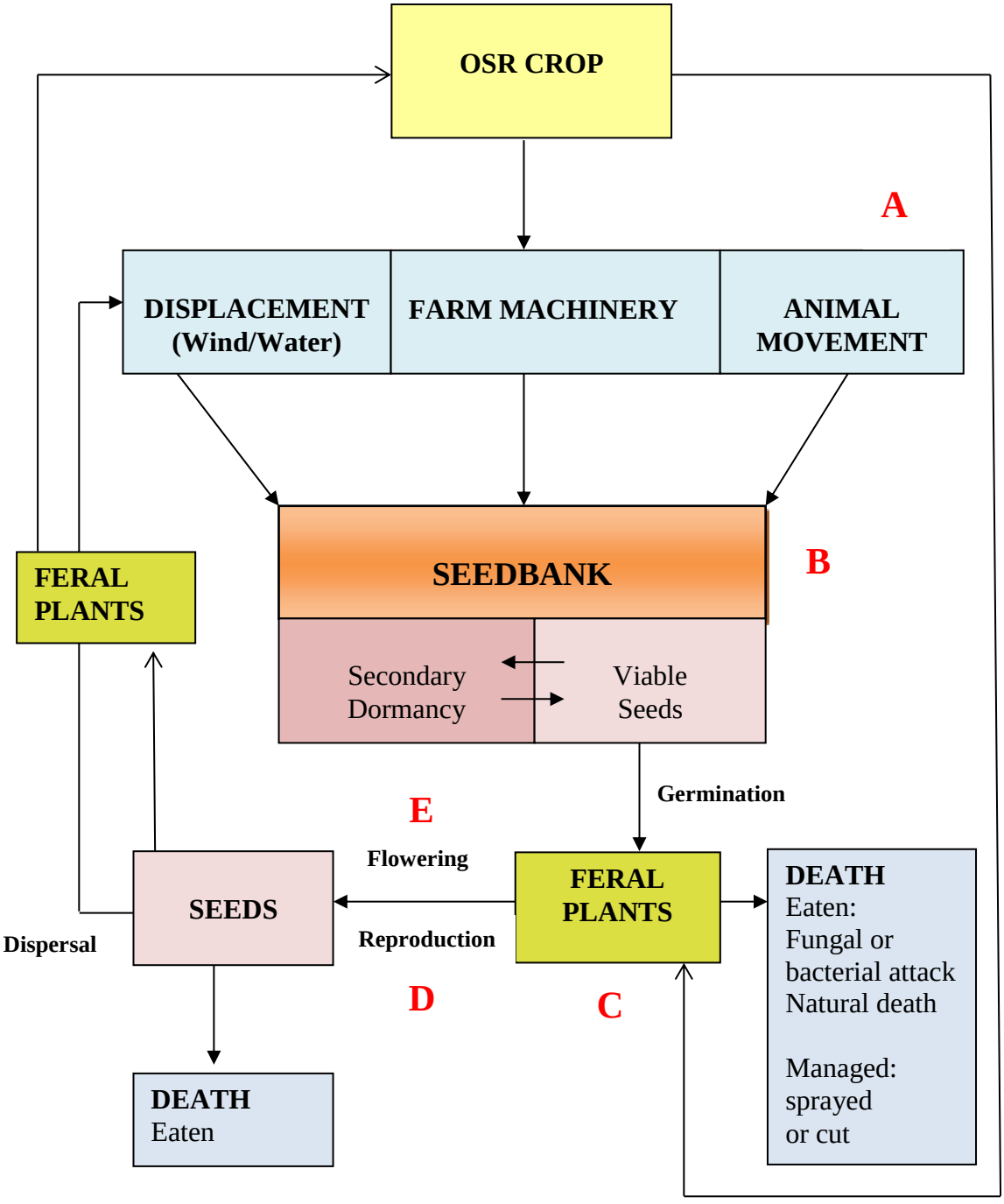


Figure 1.2 Generalized diagram of the lifecycle of feral oilseed rape

(B) The establishment of a seedbank in a ruderal environment

The ability of oilseed rape to persist buried in the seedbank strongly influences its role as a volunteer or feral. There appears to be little primary dormancy in oilseed rape. Secondary dormancy can be induced under specific conditions (absence of light, in response to water stress, oxygen deprivation or low temperatures), allowing such induced seeds to survive in soil without germinating. Squire *et al*. (2011) summarised demographic data on feral oilseed rape in different EU locations that consistently showed persistence in the soil seedbank, allowing plants to recur after an absence of a year or more. Pivard *et al*. (2008) estimated that in Selommes France, up to 40% of the observed feral populations persisted mainly through seed emerging from the soil seedbank. While seedbank populations decline year on year, there is increasing evidence that oilseed rape seedbank seeds can persist in arable soils for eight to ten years, possibly longer (Gruber *et al*. 2008; Begg *et al.* 2006; Pessel *et al*. 2001). Dormant oilseed rape has also been found in the soil seedbank of non-till systems which indicates that seed can fall dormant on the soil surface, and therefore need not be buried in the dark (Gruber *et al*. 2010). The interactions between temperature, dormancy and genotype must be examined to understand the complexities of the dynamics of seedbank populations (Begg *et al*. 2006; Crawley *et al*. 1993).

There is less direct evidence for persistence in feral habitats. Feral seedbanks are likely to contain seed from several different origins. Biochemical and molecular analyses have indicated that feral sites can contain plants with the same varietal DNA profile consistently for at least 3 years, and can contain varieties last commercially grown 3 or more years previously (Squire *et al*. 2011). Since

individual varieties of oilseed rape are sown for only a few years before being superseded by new varieties, the existence of markers from previous varieties indicates the possibility that they persisted as ferals, provided origins from farm-saved seed or persistent volunteers can be ruled out (Messéan *et al*. 2009; Pivard *et al*. 2008).

(C) Anthropological influences such as management of roadside verges, etc.

Verges in which ferals grow may be cut to prevent more aggressive vegetation from taking over. The development of scrub and trees on uncut verges can also impact negatively on the landscape, blocking views and changing landscape character. For this reason, and not specifically to control ferals, roadside verges in much of Europe are usually cut or sprayed with herbicide by the regulatory bodies. In the synthesis of ferals in five European study areas by Squire *et al*. (2011), authors reported between 25% and 95% of ferals were subject to management of some form. In a study in Scotland where in total 45% of flowering ferals were disturbed, 38% had been cut while 7% had been sprayed with herbicide as part of routine roadside management (Charters *et al*. 1999). In contrast, from observation in Scotland, ferals on wasteland or soil dumps are rarely systematically managed.

(D) Flowering followed by successful seeding and re-distribution of the feral seed

In principle, feral plants could emerge wherever seed has been spilled or deposited. Successful emergence depends mainly on the plant's ability to compete for space with primary colonizers, particularly perennial grasses. In most semi-natural vegetation oilseed rape lacks the ability to emerge due to the absence of competition free gaps (Warwick *et al*. 1999; Crawley *et al*. 1993). Therefore it emerges mostly in

habitats that are disturbed on a regular basis by mowing, herbicide applications or soil disturbance, or is replenished with seed from seed spillage from passing traffic (Simard *et al*. 2002; Pessel *et al*. 2001; Crawley and Brown 1995).

The number of feral oilseed rape plants reaching flowering and maturity is determined in part by the timing of population establishment and flowering and how many of the very young plants were grazed or otherwise destroyed. The nature of the cultivar i.e. whether spring or winter type will exert a strong influence. Spring cultivars are annual and have no vernalisation requirement (i.e. plants do not require a cold spell in order to trigger flowering and subsequent seed production). Winter cultivars have delayed flowering and produce large vegetative plants in the absence of sufficient vernalisation. Populations which flower late in the season would be less likely to produce viable seed. Local conditions such as nutrient and moisture levels will also influence seed set as well as insect pests such as pollen beetles which can also reduce the number of viable seeds produced. If viable seeds are produced then they can either re-enter the seedbank, be redistributed by animals, or be eaten.

The fate of seeding feral plants has rarely been quantified (Charters *et al*. 1999), largely because of the huge task of measuring seed deposition. However, the great capacity for seed production in oilseed rape means that if even a small proportion of ferals plants seed successfully, the population as a whole should have the capacity to increase.

(E) Geneflow by cross-pollination both into the feral population and from ferals to other members of the Brassica complex.

The main factors determining the proportion of outcrossing appear to be distance from the source and the degree of male sterility (Ramsay *et al.* 2003). Among the first studies to have looked at distance-relations in the landscape, are those of Ramsay *et al*. (2003) and Devaux *et al.* (2008, 2005) who used groups of male-sterile plants that produced no pollen of their own, to quantify the movement and impact of airborne and insect-vectored pollen grains from oilseed rape fields. They showed that cross-pollination could occur over distances up to several kilometers. In principle therefore, most feral populations growing in agricultural regions are in potential genetic contact with crops.

However, most oilseed rape is 100% male fertile - plants can self-pollinate and so the degree of outcrossing depends on the proportions of self and external pollen around the flowers. Very few measurements on male-fertile ferals have been made but estimates by Ramsay *et al*. (2003) indicate that cross-pollination to such ferals would be very low, e.g. <0.01%, except if they were located very close to a crop. Evidence of the importance of male fertility comes from work on crop varieties that are intentionally partly male-sterile (e.g. having only 20% plants that produce pollen) and in these types, (named 'varietal associations'), more than 1% outcrossing can occur from fully fertile crops within a few hundred metres (Cullen *et al.* 2008).

Some GM varieties (e.g. the glufosinate ammonium tolerant varieties used in GM crop trials in Scotland) produce some male sterile offspring on segregation, which

would depend on external pollen for fertilisation, but these plants could just as well be pollinated by neighbouring male fertile feral plants as crops.

Feral life cycle - conclusions

There is direct evidence from work on feral plants, supported by inference from work on volunteers, that ferals drop and disperse seed, form a seedbank from which they can emerge and reproduce, and set and drop seed at rates determined by the extent of wayside management and other factors. Ferals can exchange genes with oilseed rape crops and volunteers, though the extent of such exchange has not yet been quantified.

1.3 RELEVANCE OF FERAL OILSEED RAPE TO 21st CENTURY AGRICULTURE

Feral oilseed rape would have received less attention if the crop was not one of the first globally to have its range of weed control options extended by GM herbicide tolerant (GMHT) varieties. Conventional plant breeding techniques had mixed results in developing HT oilseed rape, but genetic modification techniques allowed cloning of appropriate genes from a wide variety of sources to increase the opportunities to develop HT strategies (Senior and Bavage 2003). Transgenic oilseed rape, e.g. HT and insect resistant, is now grown in many countries such as Canada and the United States of America, although it is not currently gown commercially in Europe.

The concerns about GM oilseed rape are both economic and ecological. The economic concerns arise because of the potential impurity that is caused by cross

pollination or seed transfer between ferals and crop. In Europe, if such impurity rises above 0.9%, the crop is not sellable as non-GM and would generally be destroyed. The ecological concerns arise because ferals and their hybrids with wild relatives might spread and invade semi-natural habitats, as has occurred with bent grass in North America, for example (Zapiola *et al*. 2008). The scope and results of existing studies of ferals are now summarised before examining the economic and ecological potential of ferals.

1.3.1 Studies of Feral Demography

The distribution, persistence and movement of ferals have been examined in a wide range of countries and habitats, some of which have grown or imported GM oilseed rape while others have examined the issues in advance of oilseed rape being trialed or grown. These studies differ in the extent of area surveyed and methodology involved (Table 1.2).

Table 1.2 Representative examples of the study of feral oilseed rape populations in Europe, North America and Japan, indicating period and number of years of survey, area covered, the presence of GM ferals, and whether the following were measured - genetic composition by molecular or biochemical analysis and ecological impact of ferals (e.g. on species, populations, processes)

Region	Period and years	Sampled area	Molecular analysis / introgression	GM ferals	Ecological impact	Authors
1. Discrete, contiguous study region						
UK: Tayside	1993-95 (3)	area 500-600 km^2	yes, on small fraction % of populations	no	no	DETR 1999 (Charters, Robinson & Squire)
France: Selommes	1996-97 (2) 2000-2003 (4)	area 40-45 km^{-2}	yes, on small fraction of populations	no	no	Pessel *et al.* 2001; Pivard *et al*. 2008
Germany: Bremen		area 570 km^2	no			Menzel 2006
2. Distributed plots or road segments						
UK: London	1992-1994 (2)	sampling of intermittent road segments in large scale road survey	no	no	no	Crawley & Brown 1995
Canada: Manitoba	2005-07	8 1x1 mile road segments over three landscapes		yes	no	Knispel & McLachlan 2010
New Zealand		50 3x3 km spread over wide region	no	no	no	Peltzer *et al*. 2008
USA: N Dakota	2011	sampling of intermittent road segments in large scale road survey	yes	yes	no	Schafer *et al*. 2011
3. Local area sampled in detail						
Japan	2004-07 (4)	port and surrounding roads	yes, introgression by inference	yes	no	Kawata *et al*. 2009
Canada (Quebec)	2002-05 (3)	populations around two fields	yes	yes		Warwick *et al*. 2008
Austria	1998-99 (2)	8 populations sampled in different parts of the country	SSR markers by inference	no		Pascher *et al*. 2010

One type of study, mostly in Europe, has tended to concentrate on a discrete area in which attempts were made to record all feral populations and oilseed rape fields (Pivard *et al*. 2008; Menzel 2006; Charters *et al.* 1999). A second type of study is one in which segments of road, sometimes with adjoining land, have been examined within a much wider road network (Schafer *et al*. 2011; Knispel and McLachlan 2010; Peltzer *et al*. 2008; Crawley and Brown 1995). A third type, at a much smaller spatial scale than the others, has examined the molecular characteristics of the populations and introgression (Pascher *et al*. 2010; Warwick *et al.* 2008).

This work as a whole has shown that feral oilseed rape occurs widely, wherever the crop is grown but also where it has been imported for processing; it includes GM traits if such crops are prevalent in the region; and it may persist for a few years in a locality. None of these studies have been continued for more than 4 years (except over a relatively small area in France) and none have considered any ecological impact of feral oilseed rape beyond introgression with a wild relative. Moreover the main aim of the work, particularly in Europe, has been to assess the potential contribution of ferals to impurity in crops, rather than any ecological impact.

1.3.2 Ferals in Europe - relevance to GM coexistence

In 2003 the EU commission published a recommendation to the EU member states on guidelines for the development of national strategies and best practices for the coexistence between GM, conventional and organic crops (EC 2003). At the beginning of 2007, seven EU states passed national legislation on coexistence which rose to 15 countries by 2009. Some other countries have drafts regarding coexistence or are in the process of preparing regulation. Most countries provided

proposals for separation distances for a number of crops and the differences in these values between different countries can be large. For example, maize has a separation distance of 25 m in the Netherlands but it is 800 m in Luxemburg. Different countries also have different rules regarding cropping intervals i.e. the number of years that must pass from the production of a GM crop until a similar conventional or organic crop may be grown in the same field again. It is generally thought that by using suitable separation distances the contribution of pollen dispersal to adventitious GM presence can be kept at a low level (Messéan *et al*. 2009). On the other hand, management of seed shedding and seed dispersal is difficult and requires strict management. Volunteers from the seedbank have been shown to be very important for adventitious presence in the fields (Andersen *et al*. 2010). Andersen *et al*. (2010) demonstrated that high frequencies (3.6 volunteers m^2 inter-row and up to 9 volunteers m^2 in the rows) could be found within organic row-sown fields. There is still much uncertainty as to how the occurrence of volunteers depends on dormancy, soil treatment and efficacy of control.

One of the main concerns about ferals in Europe is their role in transferring genetic information between different types of crop, e.g. high erucic and low erucic acid varieties, or GM and non-GM. As part of the EU FP6 SIGMEA project, five demographic studies in agricultural regions of Europe, including those in France, Germany (Bremen) and UK (Tayside) in Table 1.2 together with additional studies in Denmark and Germany, were used to compare the size of the flowering and seeding populations in ferals and crops, and to assess the current and potential contributions of ferals to the transfer of traits (Squire *et al*. 2011). Briefly, ferals were mapped as discrete groups of plants, or where they appeared in a much higher

density, as present or absent. Numbers of flowering plants and numbers of plants with seed were estimated. The numbers of crop plants within oilseed rape fields were estimated by multiplying the within-crop density recommended or what was typically used within a region; usually 50 to 100 m^{-2} (plants). Crop seed produced in a region was gained from agronomic and census data on mean yield and mean seed mass. Where there was any uncertainty or a range existed in the data used, the maximum estimates for feral and the minimum for crop seed were used.

The study found that the potential of ferals to introduce impurity was negligible since crop plants occurred in much greater numbers than ferals. In the extreme case that all feral seed in a region was harvested along with the crop, then the level of impurity was calculated to be between 0.001% and 0.0001% among the five study areas. A comprehensive analysis, combining these and other data, concluded that no particular management would be required to achieve impurity levels below 0.9% in non-GM crops (Devos *et al*. 2011).

Vigilance was recommended, however, since the potential for increase would remain if long-term persistence provided an opportunity for genetic recombination and stacking of traits that could give for example, tolerance to herbicides or other stresses. Under strong selection pressure, for instance if herbicide-tolerant feral genotypes were treated with the respective herbicide, evolved genotypes could increase rapidly, re-colonise fields and thereby join existing volunteer populations to increase the economic weed burden and the potential for impurity (Squire *et al*. 2011).

The minor role of oilseed rape ferals in coexistence can be contrasted with the much greater contribution of volunteers, whose populations are very much larger and grow within the crops (Messéan *et al.* 2009). More than 10% of harvested seed could be contributed by volunteers. Due to volunteers (not ferals), the conclusion of SIGMEA was that coexistence of GM and non-GM crops over time in the same field would not be possible in most of Europe.

The only uncertainty regarding the role of ferals in coexistence was whether their numbers and distribution would be likely to increase over time beyond the current levels. There is an opportunity in this thesis, therefore, to provide confirmatory evidence of feral population size over a longer period than in the studies summarized by Squire *et al*. (2011).

1.3.3 Ferals in Europe - potential ecological impact and risk assessment

One of the concerns surrounding the import (for food and feed uses or processing) of genetically modified herbicide tolerant (GMHT) oilseed rape is that the HT trait may escape into conventional agricultural or semi-natural habitats through spillage (Devos *et al*. 2012; Elling *et al*. 2009; Claessen *et al*. 2005). Also, assessing the risks associated with geneflow from GM crops to wild relatives is a significant challenge (Pascher *et al*. 2010; Kawata *et al*. 2009; Warwick *et al*. 2009; Lu 2009; Poppy 2004).

Gene-flow to non-GM cultivars and wild relatives of a crop is one of the principal routes by which GM crops could cause unwanted changes to the broader environment (Jørgensen 2007; Poppy 2004; Snow e*t al.* 1999). Because of these

types of issues, environmental risk assessments (ERA) must be undertaken before any GM crop can be accepted for commercialization in Europe.

Within the European Union, environmental risk assessment (ERA) is mandatory for a transgenic crop and post-market environmental monitoring (PMEM) is necessary for any crop intended for commercial production. PMEM aims to confirm assertions made in the environmental risk assessment regarding the occurrence and impact of potentially adverse effects of the GMO or its use, and to identify any effects which have not already been anticipated in the risk assessment (Conference - Implications of GM-Crop Cultivation at Large Spatial Scales, Bremen 2008). The European Food Safety Authority (EFSA) published revised guidelines in 2010 for the risk assessment of GMOs and derived food and feed. These documents detailed the type of scientific data that applicants must include in GMO applications and outline the risk assessment approach to be applied.

The environmental risk assessment should cover the risk to all organisms and ecosystems, including humans, exposed via, or impacted via the environment. The 2010 EFSA guidelines document describes six steps for the ERA which should be carried out on a step-by-step assessment by applicants (e.g. commercial company) wishing to release or market GMOs.

The studies in Table 1.2 indicated that very little work had been undertaken on ecological impact other than to establish that introgression can occur with wild relatives. Similarly a major synthesis of information on GM and non-GM ferals on Europe (EU FP6 SIGMEA http://www.inra.fr/sigmea/final_report) reported very

few studies of ecological impacts beyond introgression and studies of fitness in controlled conditions. There is an urgent need therefore for ecological studies in Europe to provide a background and context for ERA.

1.3.4 Potential contribution of ferals to ruderal biodiversity and food webs

The primary ecological impact of ferals is likely to be through their location in ruderal habitats. A ruderal plant is among the first to colonize disturbed lands. The disturbance may be natural (e.g. wildfires or avalanches) or due to human influences such as construction or agriculture. Ruderal species typically dominate the disturbed area for a few years, gradually losing the competition for resources to other species. If disturbance continues, however, as in many agricultural and urban areas, the ruderals may remain for long periods. So feral plants could change the plant biodiversity of an area and have concomitant effects on food webs.

Data compiled in the New Atlas of the British and Irish Flora (2003) from surveys undertaken in 1930-1969 and then repeated during 1987-1999 provided evidence that some ruderal brassica species were decreasing in number. A standardised residual for each species was used as a change index which measured the relative performance of each species between the surveys (Table 1.3).

Table 1.3 The change index of ruderal brassicas, taken from New Atlas of the British and Irish Flora (2003)

Common Name	Latin Name	Change Index	Status (Decline/ Increase)
Oilseed rape	*Brassica napus*	+ 2.88	Increase
Thale cress	*Arabidopsis thaliana*	+ 1.21	Increase
Cabbage	*Brassica oleracea*	+ 0.90	Increase
Charlock	*Sinapis arvensis*	- 1.76	Decline
Wild Radish	*Raphanus raphanistrum*	- 1.39	Decline
Shepherd's purse	*Capsella bursa-pastoris*	- 1.01	Decline
White mustard	*Sinapis alba*	- 0.90	Decline
Hedge mustard	*Sisymbrium officinale*	- 0.21	Decline
Black mustard	*Brassica nigra*	- 0.02	Decline
Winter Cress	*Barbarea vulgaris*	- 0.02	Decline

Feral and weedy oilseed rape showed the most positive change, whereas the wild, related species charlock *Sinapis arvensis* showed the most negative change. Feral oilseed rape could affect ruderal biodiversity in several ways: for example, it might reduce or displace a wild species by being more competitive; and it might compensate for the declining brassicas by providing an alternative food source for the invertebrate food web. At present, however, the ecological effect of this increase of feral oilseed rape is unknown.

Invertebrate pests

Most information about invertebrates that feed or find shelter on oilseed rape has been collected for crops. In northern Europe, oilseed rape is attacked by a wide range of insect pests, some widespread and others more limited in distribution (Alford 2003). No pests are specific to oilseed rape, but several are restricted to

brassicaceous plants, including seed crops, forage crops, vegetable brassicas and wild hosts such as charlock (*Sinapis arvensis*).

Six pests are considered of particular importance on oilseed rape in Europe, (Alford 2003): Brassica pod midge (*Dasineura brassicae*), Cabbage seed weevil (*Ceutorhynchus assimilis*), Cabbage stem flea beetle (*Psylliodes chrysocephala),* Cabbage stem weevil (*Ceutorhynchus pallidactylus*), Pollen beetles (*Meligethes* spp. especially *M. aeneus*) and the Rape stem weevil (*Ceutorhynchus napi*). Other insect pests are usually of only minor importance and are not generally targeted for pest control, and include the Cabbage aphid (*Brevicoryne brassicae*), Cabbage flea beetles (*Phyllotreta* spp.), Cabbage root fly (*Delia radicum*, now possibly becoming a major pest in Europe), Peach/potato aphid (*Myzus persicae*), Rape winter stem weevil (*Ceutorhynchus picitarsis*), and Turnip sawfly (*Athalia rosae*).

In some situations, all of the above listed species can be of some significance on oilseed rape. The two aphid species, for example, are potentially important vectors of virus diseases in winter rape crops. The larvae of the cabbage root fly and adults of cabbage flea beetles can be a problem during crop establishment, especially on spring sown oilseed rape (Alford 2003).

Little information exists on insects associated with feral plants, other than anecdotal records of the presence of a pest (e.g. Charters *et al*. 1999). However, an important question pertaining to ferals is whether they can act as secondary hosts or a *green bridge* between seasons. Pests might complete their life cycle on ferals in circumstances where their life cycle is interrupted by pest management in fields.

GM oilseed rape and the non-pest food web

Much of the information on the role of oilseed rape in supporting an invertebrate food web in farmland has come from GM crop trials. Hawes *et al*. (2009, 2003) studied the effects of GMHT (genetically modified herbicide-tolerant) and conventional crop management on invertebrate trophic groups (herbivores, detritivores, pollinators, predators and parasitoids) in beet, maize and spring oilseed rape sites throughout the UK during the Farm Scale Evaluations. It was found that the trophic groups were influenced by season, crop type and GMHT management. These studies showed the considerable contribution made by oilseed rape crops to farmland foodwebs as a whole. The oilseed rape plant itself was part of the base of this food web, but attracted specialist insects which occurred at higher numbers than on the weeds. There was no evidence that GMHT oilseed rape plants had different effects on the food web to conventional varieties: all effects in these experiments were attributed to the weed management (Hawes *et al*. 2009, 2003).

Whilst Hawes *et al.* (2009, 2003) did not measure plant chemistry and other plant quality traits, Bukovinszky *et al.* (2008) found that cultivated and feral brassicas differed in several traits including secondary chemistry, leaf thickness and plant architecture. Two aphid species, *Brevicoryne brassicae* and *Myzus persicae* (Homoptera: *Aphididae*), were found on each plant type, but there were differences in aphid body size and aphid densities which suggested that the nutritional quality of feral brassica was higher than that of domesticated brassica.

Biodiversity and food webs - questions

Feral oilseed rape has been increasing at a time when many wild cruciferous species have been decreasing. The question arises as to whether these trends are directly associated – is oilseed rape displacing ruderal crucifers? Moreover, the role of ruderal crucifers in supporting an invertebrate food web is uncertain. While oilseed rape crops can harbour many pests (Silva and Furlong 2012; Ekbom and Müller 2011; Alford 2003) and their associated weeds can act as a major resource for the farmland food web, the corresponding roles of ruderal species are largely unknown. In particular, ferals have two potential effects with respect to insect fauna: they may act as a green bridge enabling pests to complete their life cycle and persist between years; and they may influence either positively or negatively the invertebrate food web that exists on native cruciferous species.

1.4 SCOPE OF STUDY

Several questions on the persistence, spread, potential invasiveness and role in the invertebrate food web need to be addressed before the roles of feral oilseed rape can be more fully understood. Further study of these questions would benefit from being undertaken in areas where historical and contextual data already exist.

1.4.1 The contribution of the Tayside Study Area

Out of all of the demographic study areas in Europe that have examined feral oilseed rape, the Tayside region has yielded data over the longest period (Squire 2011, 1999), and covers the widest range of environments (e.g. coastal, urban land, hills, etc.). The first surveys of feral oilseed rape in this area (Charters *et al*. 1999) had the stated aims of investigating the spread of feral oilseed rape outside of agricultural

fields and the associated concerns about the plant invading natural and semi-natural habitats and exchanging genes with the non-agricultural flora. There have also been major field studies in the region of both gene flow (e.g. Ramsay *et al*. 2003) and environmental impact (e.g. Squire *et al*. 2003; Hawes *et al*. 2003) either within or closer to the area. In total, this work provides an unrivaled baseline and context.

The original feral study in this region (Charters *et al*. 1999) was over three years by surveys that comprised of (a) mapping feral and other populations over 500 km^2 countryside, and (b) close examination of the origins of, and changes in, selected feral populations. The study found significant between-year variation in population number and suggested that there might be a small rise in populations over time, but the results so far do not answer any of the main questions on persistence and spread and on the ecological roles of ferals.

1.4.2 Opportunities

The opportunity is therefore taken in this thesis to contribute to three current topics of interest and concern.

(a) Spread and persistence

One of the main deficiencies in current knowledge is whether ferals will form persistence populations where they became established and then spread into new areas. If a type of crop was grown for some years, then phased out or withdrawn, the question is whether ferals of that crop persist in its absence. The declining area of cropped oilseed rape after the mid-1990s offers a unique opportunity to investigate such potential for persistence.

(b) Coexistence

While in Europe, ferals at current population densities do not threaten the coexistence of GM and non-GM oilseed rape (Devos *et al*. 2012), the estimates of population size in Squire *et al*. (2011) for the Tayside region were based on data from the mid-1990s when the area of sown fields was high and the number of feral populations low. It is not known whether any changes in the proportion of fields to ferals at a landscape scale since then would make the achievement of coexistence more difficult. This study should provide information with which to reassess the situation.

(c) Ecological impact

The ecological role of ferals has not been formally investigated in any locality or in relation to any crop. In the Tayside Study Area, feral oilseed rape has appeared in rural and urban areas at a time when many ruderal species and weeds are declining, but its relations with these plant species and associated invertebrates are currently unknown. A specific question regarding this relation is do ferals act as more of a *green bridge* for crop pests than existing ruderal crucifers? The opportunity exists therefore for a first study of the ecological effects of feral oilseed rape in comparison to those of related wild species. Such a study would also enable a fuller consideration than has been possible to date of the EFSA guidance on the risk of persistence and invasiveness (EFSA 2010).

Structure of the thesis

This study therefore aims to address questions about feral oilseed rape and to assess its role as a model in the study of crop-feral systems. The work extends the existing demographic data on feral oilseed rape with the Tayside area of Scotland in 1993-95 to a total period of 11 years, and initiates new studies on the ecological effects of feral oilseed rape. The methods and results form the content of three chapters. Chapter 2 (Long term study of feral oilseed rape populations in Scotland: background, methodology and questions) describes the Tayside study area and summarises the changes in feral demography and population size over the 11 years from 1993 to 2004 in relation to changes in area of crop. A Geographical Information System (GIS) was used to configure spatial data of the feral oilseed rape and crop fields within a region of around 500 km^2. The main question to be addressed is whether the number and size of feral populations changed over this period in the face of the decline in area of land sown with oilseed rape. Particular attention is given to the occurrence of later flowering 'spring type' ferals following the decrease of the spring crop to near zero by the end of the period.

Chapter 3 (Spatial and temporal change in the Tayside long term study area in Scotland) examines the spatial configuration and persistence of the feral populations over the period, especially in relation to the proximity of fields previously and currently cropped with oilseed rape. The main hypotheses to be examined concern (a) persistence and spread, for example, the extent to which ferals persist in the same locations over time and whether they have spread beyond the main transport corridors, and (b) isolation and connectivity, notably whether they have become closer to or farther from fields over the period.

Chapter 4 (Comparison of performance and function of feral oilseed rape and charlock) examines more directly the potential ecological impact of the ferals. It compares feral oilseed rape and naturally occurring *Sinapis arvensis* (charlock), in terms of attributes such as plant biomass and role within the food web for both pest and non-pest invertebrates. Charlock was chosen as a comparison to oilseed rape because it is a closely related crucifer, and occurs in similar ruderal locations to oilseed rape. Charlock is an archaeophyte (an old but introduced species, whereas oilseed rape is a new introduced species) and is declining, so there is a potential for oilseed rape to replace it, compete with it for resources, or substitute for its role in the ruderal food web.

Finally, Chapter 5 (Contribution of the work to feral demography and environmental risk assessment) considers the results in Chapters 2, 3 and 4 in relation to the increasing literature on crop ferality, and in particular assesses the role of a long term demographic study as a baseline in the environmental risk assessment of GM crops (EFSA 2010). To these ends, this study aims to provide a greater understanding of the potential ecological and economic roles that feral oilseed rape and other crop-derived feral plants might have in current and future agriculture.

2. Long term study of feral oilseed rape populations in Scotland: background, methodology and questions

2.1 INTRODUCTION AND CONTEXT

The original three year project that established the Tayside Study Area aimed to assess whether feral oilseed rape would pose an ecological risk if GM oilseed rape crops were commercialised in the UK (Chapter 1.4.1; Charters *et al*. 1999). This work described aspects of feral population dynamics and commented on the potential origin of some of these populations, but could say little about persistence or invasiveness due to the short-term nature of the observations.

Feralism has since remained high on the agenda in considerations of environmental risk assessment both in Europe and elsewhere (Schafer *et al*. 2011; Knispel & McLahlan 2009; Kawata *et al*. 2009) and has more recently entered the debate on GM coexistence (Messean *et al*. 2009). The EFSA (European Food Safety Authority, 2010) document providing guidance for the environmental risk of genetically modified plants emphasised the need for relevant long term datasets on demography and ecological impact. The potential of the Tayside study area to provide such long term data on the spread and impact of oilseed rape is examined here. This Chapter describes the study area, previous research, and the methods used for the investigations. It also undertakes preliminary analysis of the main changes in fields and ferals over an eleven year period from 1993-2004.

2.1.1 The *Brassica* complex in the Tayside Study Area

Most components of the *Brassica* complex (see Chapter 1.2) are present in Tayside. The crops consist of oilseed rape (*Brassica napus*) which is the main yellow-flowering oilseed; turnip rape (*Brassica rapa*) a minor oilseed, grown until the 1990s but rarely since; *B. rapa* 'root' crops; and cabbage (*Brassica oleracea*) in a range of cultivated forms. All three species are used variously for human food and fodder and to a lesser degree for industrial feedstocks (e.g. high erucic oilseed rape). The areas occupied by these various forms are not recorded separately in the annual agricultural census. The oilseed forms are classed as 'winter' or 'spring' oilseed rape, so no records are available that show the extent to which, for example, *B. rapa* oilseed was grown in preference to *B. napus*. The vegetable *Brassicas* are grouped together with other vegetables, which as a whole have never occupied more than a few percent of the arable surface. In 2012 oilseed rape comprised 6.5% of the arable area and the combined vegetables comprised 7% of the arable area (www.scotland.gov.uk/publications/2013/06/5219/5).

Volunteer weeds are mainly *B. napus* and occasionally *B. rapa*. No volunteer oilseed rape was found in the seedbank (2.1.2 below) before the 1990s, despite the crop being widely grown in the 1980s, but by the time of the Farm Scale Evaluations in 2000-2003 (2.1.2), it was among the top ten most common species of the arable seedbank (personal communication: G.R. Squire, data for sites in Scotland from the Farm Scale Evaluations data archive). Debeljak *et al*. (2008) analysed data from 2000-2003 and found that the highest abundance of volunteer oilseed rape in the UK was in the north-east of Scotland.

Volunteer oilseed rape forms a large, persistent seedbank, produced from seed drop at harvest that can be as high as 10% of the yield (personal communication: G. R. Squire).

Its seed can survive temperatures below freezing and it begins to germinate when temperatures increase above 0^{o}C (Squire 1999). This, combined with secondary dormancy (Marshall *et al*. 2000; Marshall & Squire 1996), generally mild winters (compared to continental climates of Europe) and the prevalence of inversion tillage make the agronomy and climate ideal for its growth and persistence. The seedbank declines over time but there is evidence that it can persist for at least 8 to 10 years (Squire *et al*. 2012). There are no special weed control measures directed at volunteer *B. napus*; it is managed along with other broadleaf weeds, such as *Veronica persica* and *Capsella bursa-pastoris*, by herbicide, cultivation and cropping sequence.

No attempt to record ferals of the *Brassica* complex were made before the 1993 survey (see Chapters 1.4.1 and 2.2 below). Ferals are mainly *B. napus* (Charters *et al.* 1999). Transient roadside populations of *B. rapa* and *B. oleracea* were found in the region during the 1993-1995 surveys but are rare (SCRI archive records). Populations ranged in size from one plant to stands of over 1000 and mostly flowered during the early 'winter' oilseed rape flowering period. They occupied three broad habitats: roadsides, field margins and agricultural soil tips, but more than half the populations occurred on roadsides (Charters *et al*. 1999). Feral populations were not found in natural or semi-natural habitats, in planted forest or woodland.

The main omission from the *Brassica* complex is wild *B. rapa*. The *B. rapa* shown in Scotland on the map in Preston, Pearman & Dines (2002) is likely to be crop-derived and not of the type that exists along river banks in England (Wilkinson *et al*. 2003). Of the other wild relatives, *B. oleracea* exists on the cliffs and rock ledges of the east coast. Wild radish (*Raphanus raphanistrum*) occurs as a weed in fields and has the potential to

hybridize with other members of the *Brassica* complex (see Chapter 1). Several species related to *B. napus* but not sexually compatible with it, grow as weeds in fields and ruderals on roadsides and waste land: the most common of these are *Sinapis arvensis* and *Sysimbrium officinale* (Hawes *et al*. 2010; Heard *et al*. 2003).

2.1.2 Previous studies involving the *Brassica* complex in Scotland

Many studies of the biology, genetics and ecology of the *Brassica* complex have been undertaken in and near to the areas (Ramsey *et al*. 2003; Charters *et al*. 1999; Squire *et al*. 1999) that constitute the main surveys of ferals described in this thesis. As a whole, this body of work provides a valuable background on life cycle biology, geneflow, population dynamics and associated plant communities and food webs that is highly relevant to the analysis of ecological impact described in later chapters.

Seedbanks and weed communities

Seedbanks of arable and grass fields have been studied at the Scottish Crop Research Institute near Dundee since the 1980s (unpublished, SCRI archive records). Seedbank communities tend to be high in abundance: in intensely managed cereal rotations they ranged from 2000-5000 m^{-2}, and in areas of low-input farming they can reach 10,000 m^{-2} (Hawes *et al*. 2010). The number of species in a field ranged from around 10 under high intensity management to 40 or sometimes more under low intensity management (Squire *et al*. 2009). Among the most abundant broadleaf weeds are *Sinapis arvensis* and *Capsella bursa-pastoris*, both of the Crucifer family and similar in habit and form to volunteer oilseed rape. As indicated above (2.1.1), oilseed rape is found in the arable seedbank at abundance similar to these other Crucifers.

GM crops trials

Several commercial GM crop trials were sited in east Scotland in the 1990s. One of them, trialling an oil-modified variety of *B. napus*, was used as a potential pollen source by Ramsay *et al*. (2003). In the years 2000-2003, seventeen sites within the Farm Scale Evaluations of Genetically Modified Herbicide Tolerant oilseed rape (Squire *et al*. 2003) were established in east Scotland. One site was in Fife, just to the south of the main feral study area, and the rest were in Aberdeenshire and Moray, well to the north. None of the sites were within the study area, and the size of these GM fields occupied a very small proportion of the total oilseed rape in the region, where stringent practices were in place to prevent spillage during transport, resulting in a negligible chance that GM ferals occurred in the area alongside non-GM ferals.

Cross-pollination and geneflow

Pioneering studies in landscape-scale geneflow were carried out by SCRI in agricultural landscapes of east Scotland during the late 1990s. Ramsay *et al.* (2003) examined gene flow in oilseed rape in three areas, which included The Carse of Gowrie, east Perthshire and the western fringe of Angus, by using male-sterile plants as pollen recipients. Later, such work was extended to Aberdeenshire. This study found that pollen was carried many kilometres on a range of insects and in the air. Factors such as weather, pollinator populations and relative strengths of competing sources of pollen were found to affect levels of cross-pollination. However, the main factors determining cross pollination were distance from the source and degree of male sterility in the recipient.

2.1.3 Aims of the study

None of the existing work undertaken in Europe or Scotland can answer issues surrounding feral oilseed rape in terms of invasiveness, persistence and impact (see Chapter 1.4.1). The early study of ferals in Tayside provided context and background but was inconclusive as to persistence and invasiveness. However, an opportunity was provided to extend the study through the EU FP-7 SIGMEA project, which began in 2002 and reported in 2006/07 (Messéan *et al*. 2009).

This thesis combines the earlier data (1993-1995) with subsequent unanalysed raw data, along with the data collected with funding by SIGMEA during 2004 and 2005. The work described in this chapter aims to define the main changes in cropped area and feral populations over the period 1993 to 2004, and to specifically investigate:

- whether feral populations changed in number as the area of crop declined;
- whether populations remained concentrated along main transport routes as they were during 1993-1995;
- if the number and distribution of feral populations were likely to have a potential economic impact through the need for coexistence measures and an ecological impact through invasiveness or impact on ruderal communities.

2.2 DATA SOURCES AND METHODOLOGY

2.2.1 Sources of data on ferals and fields in the study area

The basic information on fields and ferals was brought together from three sources. The first was the 1993-1995 survey conducted by E. O'Brien, Y. Charters and A. Robertson, latterly supervised by G.R. Squire, covering an area of more than 500 km^2 which

included Angus, The Carse of Gowrie and part of Fife (Charters *et al*. 1999). The Fife area was dropped after the first year since it provided no additional habitats to those already covered in the other areas. The Angus area consists of mainly farmland between the towns of Dundee, Arbroath and Forfar, bounded by transport corridors and coastal land. The Carse is a strip of land to the west of Dundee between the Tay estuary and the Sidlaw Hills to the north (Charters *et al.* 1999). The areas in Angus (>500 km^2) and The Carse (25 km^2), with the city of Dundee in between, are here together referred to as the Tayside Study Area.

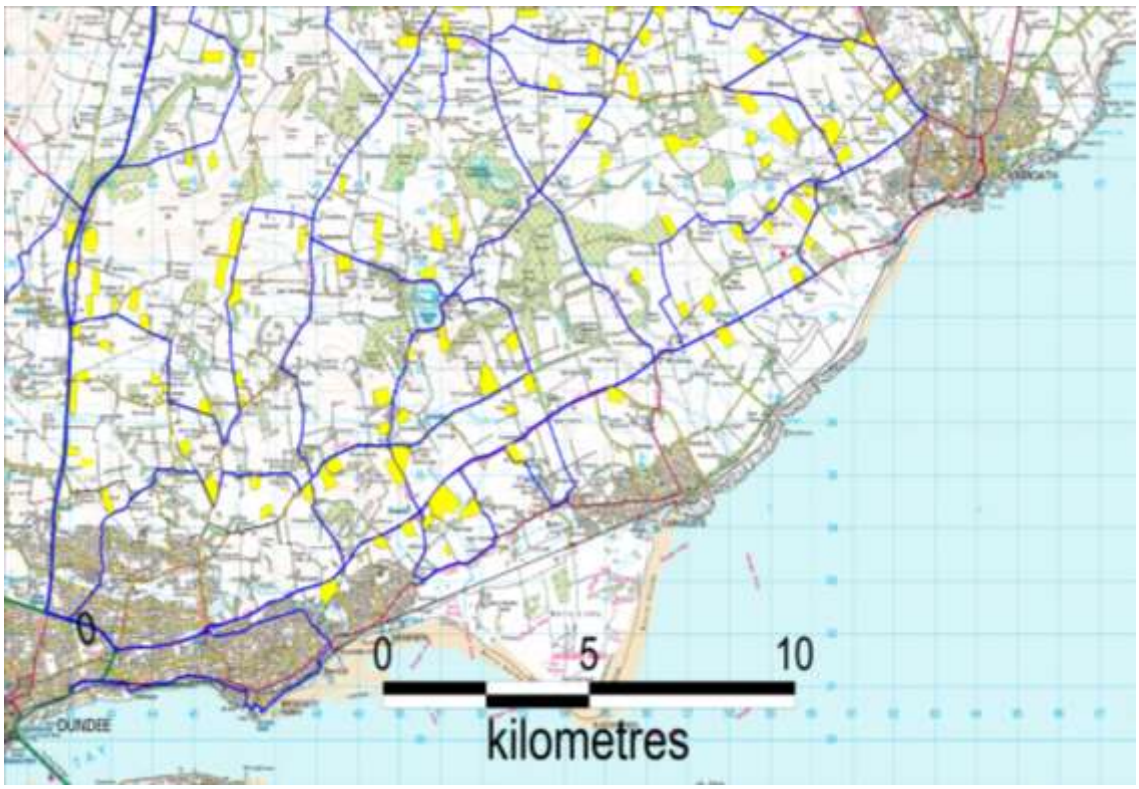


Figure 2.1 Map showing the location of the road network (blue lines) and representative oilseed rape fields (yellow polygons) in the Angus area - Dundee to the lower left and Arbroath to the right

O'Brien, Charters and Robertson (1993-1995) established much of the methodology and provided data both as a hard copy and as spatially mapped locations on early versions of MapInfo software. An example of the road network followed during the survey and representative fields, in the Angus area is shown in Figure 2.1.

The second source was 'hard copy' data from work over the period 1996-2000. A main survey following the exact route and methodology as the earlier surveys, was conducted in Angus and The Carse of Gowrie in 1996 by SCRI's field staff, including G. Wright, again under the supervision of G. R. Squire. In addition, occasional surveys over the period 1998-2000 were conducted in Angus and The Carse by G. R. Squire over parts of the area and are used here only to provide additional background. The third source was the survey in 2004 undertaken in Angus by G. Banks (author), following identical procedures to those used earlier.

G. Banks checked and audited all original data from 1993, 1994, 1995, 1996 and 2004 and re-entered it consistently into a digitalized GIS system. Following standard practice, all data were double-checked by colleagues, notably Mark Young in this instance, before all surveys were 'signed' off by G. R. Squire, according to standard practice.

An additional demographic survey was undertaken in 2005 as part of a 3-region comparison (France, Germany, UK) within the SIGMEA project. The work in the UK followed a similar foot and road survey as described earlier, but involved three differing areas in Angus and The Carse, each about 40 km^2. All fields, ferals and wild relatives were recorded and their description and habitats described. This information from the

three areas is not considered further in this chapter, but was developed for use in the ecological studies described in Chapter 4.

All of the information was processed using GIS (Geographical Information System – MapInfo Professional 10.0, 2010, Pitney Bowes Software Inc.) which was used to locate the feral population and fields, to calculate the area of fields (see 2.2.3), to estimate distances and to define attributes such as road type and altitude. The GIS allowed the large datasets to be effectively visualised and summarised.

2.2.2 Description of the study area

The Tayside Study Area lies within the counties of Perthshire and Kinross and Angus, which along with the City of Dundee, cover an area of around 7500 km^2 (750 000 hectares). The farmland within this area stretches from mountains, hills and glens, through fertile valleys and straths to the carselands of the coastal plains and estuaries (www.angus.gov.uk/biodiversity/pdf). This area has been influenced by man from the earliest forms of agriculture just over 5000 years ago to the present day. Arable land accounts for just over 2000 km^2 of this area, contributing to the patchwork of fields, hedges, dykes, veteran trees and farm buildings that are generally associated with land under cultivation in maritime regions (www.angus.gov.uk/bidiversity/pdf). Much of the rest of the area is managed and unmanaged grassland.

Combinable crops (malting barley, winter wheat, oats and oilseed rape) are the mainstay of the area's agriculture, accounting for 98 000 hectares. A portion of the low lying agricultural land, a further 86 000 hectares, is under rotational grassland, used for grazing sheep and cattle or for producing hay and silage for winter feeding. Other major

crops include potatoes (11 000 hectares), vegetables for human consumption (3 500 hectares) and over 1 500 hectares of raspberries and strawberries.

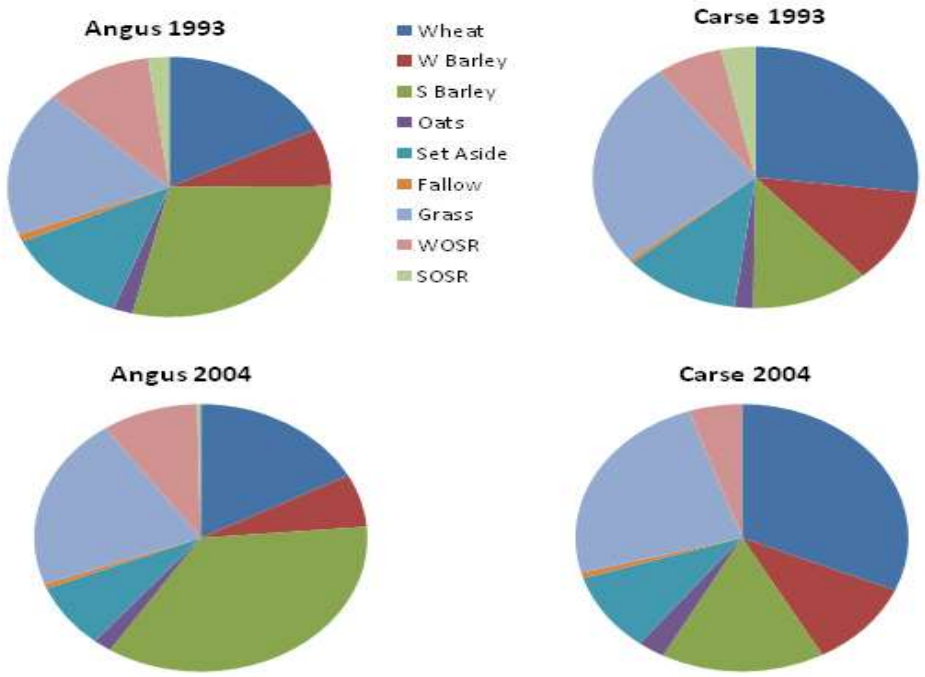


Figure 2.2 Major crops grown in Angus and The Carse in 1993 and 2004

The hectarage and proportions of land area devoted to each of the main crops grown in the Angus and The Carse areas changed between 1993 and 2004 but the same types of crop were grown throughout the period (Figure 2.2). The Carse generally has more winter wheat and less spring barley than Angus, but otherwise grows similar crops.

Natural boundaries and transport routes

The Angus area is delimited by hilly land to the west and north and low-lying or coastal land to the south and east. Two main transport corridors, formed by dual carriageway (trunk roads) pass through the area: one north-south, running from Dundee in the direction of Aberdeen; and another east-west from Dundee to Arbroath a few km inland

from the south coastal boundary. A lesser corridor formed by A and B roads runs east-west near the northern boundary. The majority of the area consists of farmland served by minor roads and tracks, ranging from land near sea level to the south and rising to an altitude of 200 m in the centre and north. Corridors and farmland are defined more strictly and considered later (2.3.2) in relation to the density of fields and ferals.

The oilseed harvest is taken from the area by two main routes – to the port of Dundee and from there to elsewhere in the UK or overseas, and to processing plants in the south, mainly in England (personal communication from a range of farmers). No easily accessible information is available on the proportion of the harvest that goes by these routes. Individual farms may aggregate the harvest from different fields into a silo or temporary store, but in general different owners or contractors do not use a common silo. The situation is therefore different from that in the Selommes study area in France (Pivard *et al*. 2008) in which there is a central silo collecting seed from many farms. Oilseed from other regions, such as Aberdeenshire to the north or Perthshire to the west moves along the major trunk road running north-south to Dundee and then west to Perth and onwards, therefore by-passing most of the farmland in Angus and The Carse. Most of the oilseed carried by vehicles through the study area therefore has an internal origin, that is, harvested seed is taken from fields, sometimes to a temporary store, and then directly to the port or along the trunk road out of the area.

Areas of oilseed rape

The data accessible from the annual government census of agriculture (Chapter 1.1) is on too coarse a scale to show trends in the Tayside Study Area. Therefore, the areas of winter and spring oilseed rape were requested from government statisticians for groups

of agricultural parishes making up as closely as possible those parts of north and south Angus and The Carse that fall within the survey.

Trends over time in the area of oilseed rape are quantified in Figure 2.3 which indicates a decline in the area of both winter and spring fields.

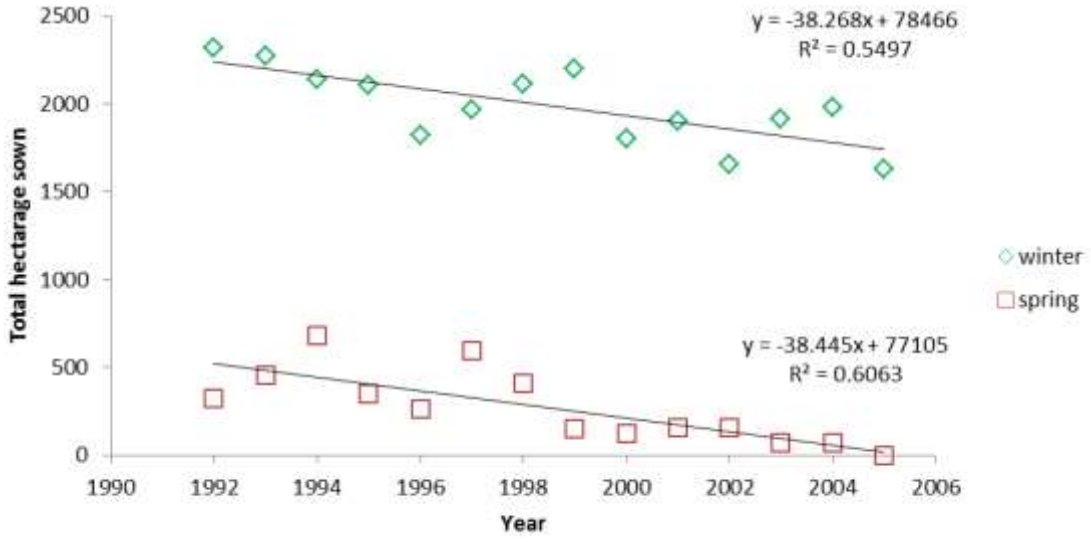


Figure 2.3 Trends in area of cropped oilseed rape fields (1992-2005) within The Tayside Study Area from data provided by government statisticians.

The fluctuation in area sown to oilseed rape in any given year may be due to agronomic and economic decisions – for example spring oilseed rape is generally grown by default after the failure of an autumn sown crop, or as part of a crop rotation, which allows some flexibility in the rotation if preceding a late harvest which is followed by poor weather conditions. The marked decline in the spring crop was caused by several factors. In the 1990s changes were made by the regulatory authorities that stated spring oilseed rape no longer needed to be brought to seed before subsidy payments were

made. This allowed farmers to sow spring rape on poor ground but remove it well before harvest for fodder. After this rule was revoked in the late nineties many farmers lost interest in growing spring oilseed rape because although the crop has a shorter growing period than winter oilseed rape, it is more sensitive to the weather than winter rape and yields less. Winter oilseed rape is used as a profitable break crop for cereals where it allows wheat and barley to be positioned as first crops in a rotation where they yield far better than if they follow other cereals. Generally when oilseed rape prices are high at market then there is an increase in the area planted relative to cereals.

2.2.3 Survey Methods

Full details of the survey methodology are given by Charters *et al*. (1999). The same road network was followed on each occasion. Frequent stops were made to enable sighting of populations and identification by binoculars if required of all fields of oilseed rape in the area. Surveyors inspected on foot all feral sightings and walked along farm tracks to cover a large part of the potential habitat for ferals. The surveys were conducted to coincide with the flowering periods of winter sown and spring sown oilseed rape, typically in May and June/July, respectively.

Recording oilseed rape feral populations

At each occurrence of a feral population, the location was recorded and the number of plants counted. Coordinates were taken using a Trimble dGPS in 2004/2005 (in previous years the positions of ferals were recorded on a paper map). Habitat (e.g. roadside grass verge, field margin, soil tip, pavement, etc.), reproductive state (e.g. bud, flowering or producing seeds) and plant height (into categories small <50 cm; medium 51-100 cm; large >100cm) were noted.

Ferals tended to occur mostly as individuals or in distinct groups at definable loci that were well separated by tens to hundreds of metres from each other. Occasionally, loci were indistinct, such as when plants occurred more or less continuously along linear features. In these instances, loci were considered distinct if they were 10 m or more apart. In the early work on the survey, the plants at each locus were termed a 'population', and this term is used widely in European work on feral oilseed rape to mean a locus or position at which feral plants exist. For consistency with this convention, the plants at a locus in the surveys are termed a population (which may be of one or more plants), but this name implies no particular biological or genetic characteristics of the plants at any locus.

Feral populations were grouped for comparison across the years into abundance categories since it was not always possible to get exact numbers of plants in a population (for instance, if the population was large and in the middle of a busy road.). Populations were therefore grouped as follows: 1= 1 plant; 10 = 2 - 10 plants; 100 = 11 - 100 plants; 1000 = 101 - 1000 plants; >1000 = more than 1000 plants.

Wild relatives and other wild crucifers were noted if they occurred within a feral population, along with a written description of the location in which it had been found. Some wild crucifers were found to co-habit the same area of land as feral oilseed rape, in some instances being less than 50 cm apart.

Flowering feral populations that occurred around the same time as winter sown oilseed rape crops were deemed ‘early’ feral populations, and those that occurred around the same time as spring sown oilseed rape crops were called ‘late’ feral populations.

Reproductive state was classed as bud, flower, or pod. The predominant state covering most of the population was indicated.

Oilseed rape fields in flower

Oilseed rape fields in flower at the time of the survey were recorded on paper maps, drawing the extent of the crop in relation to natural boundaries. Cropped areas were then located on the digitised Ordnance Survey 'base map' which showed fields and boundaries. Those flowering in the early (May) period were likely to be winter oilseed rape varieties sown in the previous autumn (September/October), while those in the late period (July) were spring oilseed rape varieties which would likely have been sown in April.

Each field was given a unique ID number and identified by the coordinates at their centroid or edge as appropriate. Each field was registered as a polygon whose area was estimated through the MapInfo software. In total, thousands of fields were mapped and quantified in this way during the study.

2.3 RESULTS OF THE SURVEY – CHANGE IN FIELDS AND FERALS

2.3.1 Number and area

Change in fields and ferals are presented here for the Angus area. Table 2.1 summarises the main changes. The Angus study area is defined by the road route of the survey plus a distance outside the route that was viewed for the presence of fields and ferals. The total area is defined 556 km^2. The total area of fields was just less than 2500 ha in 1993, consisting of approximately one quarter spring crop and three-quarters winter crop. The areas of both winter and spring crops declined over time in a way that was largely

consistent with the government census data in Figure 2.3. Notably, the spring crop had declined to only a few hectares by 2004.

Table 2.1 Demographic change in fields and ferals in the Angus area

		1993	**1994**	**1995**	**1996**	**2004**
total area of fields (ha)	winter	1876	1869	1781	1397	1623
	spring	585	714	354	290	45.9
	all	2461	2583	2136	1687	1669
feral population number	early	57	37	126	229	306
	late	20	38	33	47	55
	all	77	75	159	276	361
population per unit study area (km^{-2})	early	0.102	0.066	0.226	0.411	0.550
	late	0.036	0.069	0.059	0.084	0.099
	all	0.138	0.135	0.286	0.496	0.649
population per unit crop area (km^{-2})	early	3.04	1.98	7.07	16.38	18.84
	late	3.42	5.32	9.32	16.21	119.83
	all	3.13	2.90	7.44	16.36	21.62

In contrast, early and late ferals both increased over time (Table 2.1). Early ferals increased 5.37 times between 1993 and 2004 and lates 2.75 times. Expressing populations as the number per unit area of the survey shows a low mean frequency, always less than 1 km^2 of land throughout the period. Expressed as number of populations per unit area of crop, however, the lates increased due to a large fall in crop.

The new data presented for 1996 and 2004 show evidence of a major trend of increase in time. The switch to a higher percentage of lates than earlies in the second year, 1994, may have obscured a possible increase suggested by the high value of earlies in 1995. By 1996, however, the increase in earlies was clearly evident, and confirmed by the

survey in 2004. Much of the rise had already occurred by 1996. To assess whether the trend was widespread across Angus, or confined to the main through-routes the region was sub-divided into transport corridors and general farmland.

2.3.2 Feral abundance in transport corridors and farmland

The Angus region was divided into five sub-regions to assess whether the greater occurrence of ferals along transport routes continued into later years. Three transport corridors were defined as the main N-S route of the A90, the E-W route of the A92 near the coast and the E-W route further inland, near the northern extremity of the region. The lateral extent of the transport routes was taken arbitrarily to be approximately 1 km either side of the main road. The rest of the area was designated ‘farmland’ and divided into that mostly to the south of the region and mostly below 100 m (farmland – low) and that to the north and west and mostly above 100 m (farmland – high) (Table 2.2). The percentage of the area sown with oilseed rape crop was similar among the regions, indicating a generally similar agricultural land use: the difference was mainly that a main road ran the length of the corridors, while only minor roads and tracks permeated farmland.

Table 2.2 Characteristics of three transport corridors (1, 2, 3) and two farmland categories (4, 5) of Angus in terms of area, oilseed rape crop (winter and spring combined) as a percentage of area, percentage of all ferals in all years, and the frequency of feral populations estimated as number of ferals (early and late combined) divided by area

ID	corridor or farmland	area (km^2)	crop (% area)	% all ferals	frequency (km^{-2})
1	corridor N-S (A90)	48.2	4.90	15.3	0.59
2	corridor E-W coastal (A92)	36.9	5.03	19.1	0.96
3	corridor E-W inland (A932)	43.0	4.73	9.7	0.42
4	farmland low <100 m	108.7	3.03	28.1	0.48
5	farmland high > 100 m	319.4	4.21	27.8	0.16

More than half (56%) the feral populations occurred in areas designated as farmland, which comprised 77% of the total area. The transport routes covered only 23% of the total area, yet contained 44% of the total ferals (all years, early and late not distinguished). To account for the different areas of land, feral populations were expressed as a frequency (number per square kilometre per year) which were were then used to show differences between years and between early and late feral populations.

Frequencies for the three corridors combined and two farmland areas combined are shown by year in Figure 2.4. Consistent with Table 2.1, the frequencies were low in 1993 and 1994 then increased greatly with each sampling year. Each increase in the corridors was matched by a proportionate increase in the farmland, but the frequency was always higher in the corridors - at the mid-point of the range in Figure 2.4, the frequency in corridors was 2.5 times that in farmland.

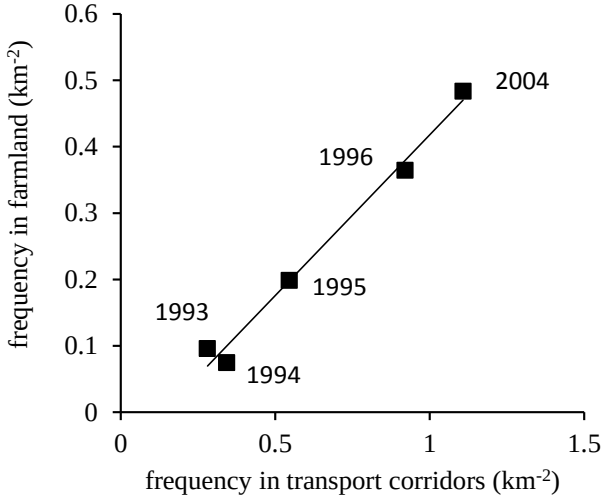


Figure 2.4 Comparison of number of feral populations per unit area (frequency) in transport corridors and farmland, year of measurement indicated; the line is y = 0.48x – 0.067, r^2 = 0.98

The frequencies for the five sub-regions were estimated separately for early and later ferals (Figure 2.5). Despite a six-fold range in frequency between sub-regions, the proportion was similar among all sub-regions; the mean ratio of late to early was 0.26.

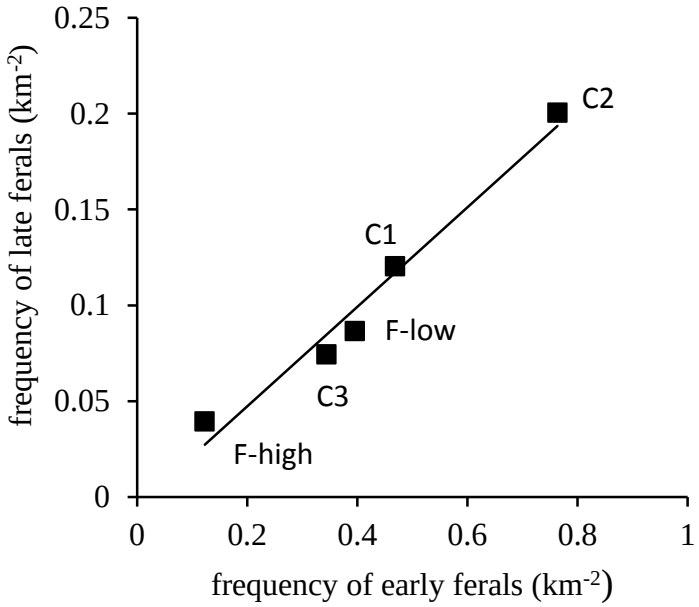


Figure 2.5 Comparison of the frequency of early and later feral populations in the five sub-regions: corridors C1, C2 and C3 and farmland F-low and F-high (Table 2.1); the line is y = 0.26x, r^2 = 0.97

While a systematic trend over time is evident after 1993 and 1994 (Table 2.1, Figure 2.4), the trend was not the same in all sub-regions. For those more inland than coastal (1, 3 and 5 in Table 2.2), the linear trend, estimated by regression of population number (y) on years (x) was and highly significant, as follows: corridor N-S, y = 0.064x - 127, r^2 = 0.99; corridor E-W inland, y = 0.67x – 133, r^2 = 0.99; farmland–high, y = 0.029x – 59, r2 = 0.90. For the coastal corridor and farmland (2 and 4 in Table 2.2), the regressions were not significant; the rise in number reached a maximum in 1996, then either fell (corridor E-W coastal) or remained the same (farmland-low) in 2004. (These differences in population growth and dynamics within Angus are examined in Chapter 3).

2.3.3 Population size

Major changes also occurred over time in the proportion of populations of different size. In 1993, about half the populations were in the larger two categories, either containing 10-100 plants or 101-1000 plants. By 2004, numbers of populations in both these categories increased but they were proportionately reduced to 23% of all populations. The shift in population size was significant when assessed by a chi-square test for early ferals and but not significant for late (Table 2.3). Over time therefore, the number of populations increased but relatively more of them were small populations.

Table 2.3 Number of populations in abundance categories (1= 1 plant, 10 = 2 to 10, 100 = 11-100, 1000 = 1001-1000) over five sample-years for early and late ferals

Contingency tables and chi-square test: early ferals, 12 d.f, chi square value 55.2, probability level (under null hypothesis) P<0.001; late ferals, 12 d.f., chi-square value 20.85, P = 0.053 (ns)

Type	**year**	**population size**			
		1	**10**	**100**	**1000**
early	1993	14	15	22	6
early	1994	11	14	12	0
early	1995	14	57	42	8
early	1996	55	112	59	3
early	2004	90	144	50	18
late	1993	4	5	7	4
late	1994	8	15	10	5
late	1995	9	10	11	0
late	1996	14	22	11	0
late	2004	12	21	17	5

Several populations containing more than 1000 individuals were found in two of the years and are not included in Table 2.3. It can be argued that such populations should not be categorised as ferals where they occur in derelict fields or as a result of an influx of soil at major construction sites. They would be clearly visible and can be readily controlled if necessary, but they have been considered in the following analysis.

Proximity of large populations to fields

Large populations in categories 101-1000 and >1000 have particular relevance to the coexistence of different types of crop in a region, since they might constitute opportunities for substantial gene movement by seed or pollen to nearby fields. These large populations, of which there were 59 in all years, occurred throughout the area and were not confined to the main transport routes. Most were early flowering, at the same time as the winter crop and only 6 flowered late, at the same time as the spring crop. Three of these large populations in all five years occurred adjacent to a field, defined as being within 10 m of the field. Only 20% of the 59 the populations were within 100 m from a field.

These populations can be compared with the typical population size of oilseed rape plants in a field. The mean area of fields in this study was 7.6 ha, and most fields were between 5 and 10 ha. The typical, recommended stand density (confirmed from field observations in the region) is 40 to 60 m^{-2} at flowering. Therefore, taking the smallest and largest combinations of the above, the total number of plants in a field would range from about 2 million to 6 million. If impurity were estimated on the basis of number of plants, and plants in fields and in feral populations produced seed similarly, then a 0.9% impurity (the threshold for coexistence) would be equivalent to 180,000 and 540,000

plants for the ranges of population density and field area cited. None of the feral populations was large enough to be within 10% of these values, and most of the large populations were <1% of these values.

2.4 IMPLICATIONS AND FURTHER QUESTIONS

The three questions posed at the beginning of this chapter are now considered: (1) how did feral populations change as the area of crop declined; (2) were they concentrated along transport routes or did they also spread into farmland; and (3) would they constitute a risk. The third question is divided into an economic risk through the need to implement measures to manage coexistence and an ecological risk through a potential for invasiveness or impact on ruderal communities.

2.4.1 Feral population number

The feral populations increased rather than just maintained their number during the period when the area of crop declined. The abundance of all population categories increased over time, but there was a shift towards a higher proportion of small populations. Early ferals, flowering at the same time as the winter crop, were the most common form, but late ferals also increased and remained in a ratio of 1 late to 4 early feral populations. This chapter therefore presents the first evidence of increase over more than a decade in feral oilseed rape over a large area consisting of both transport corridors and farmland.

The possible cause of these changes merits further examination, but would need to take into account the existence of a persistent feral seedbank containing seed of different origin (Beckie and Warwick 2010; Pekrun *et al*. 2005). The seedbank can gain seeds by

either reproduction of feral plants which then drop their seeds at the same location, or by new seed being bought in from surrounding areas, such as on the wheels of agricultural vehicles. Seed can also be lost under certain agronomic conditions which may include attack by pathogens, herbivores, or death after germination due to adverse weather or agronomic control (Squire *et al.* 2011). Theoretical studies have identified survival in the seedbank as the life history trait with the largest impact on population growth rate and persistence (Pascher *et al*. 2010; Claessen *et al*. 2005). In a study in France, the origin of at least 35% of feral oilseed rape populations were deemed to come from the seedbank, i.e. arising from seed shed in a previous year and persisting in the soil (Pivard *et al*. 2008). A feral population growing in a given year could therefore arise from immediate immigration, for example dropped from vehicles or transferred from neighbouring fields or from seed deposition in a previous year either at the location in question or by redistribution from another location. An examination of the characteristics of persistence and spread form a major part of Chapter 3.

Early and late ferals

Early ferals recorded in this study would not have had time to germinate in spring and develop to flowering at the same time as the winter crops; they would have germinated in the autumn and overwintered. In contrast, late ferals would have germinated in the spring. The morphological characteristics of the respective plants were consistent with the different germination times, early ferals being larger in stature and generally with a basal rosette.

However, it is not certain from morphological traits alone that all early ferals originated from winter varieties and late ferals from spring varieties. Despite procedures by plant

breeding companies to ensure uniformity, a single crop variety grown in the 1990s could contain traits leading to a range of time to flowering Squire (1999). Molecular methods can be used to distinguish crop varieties, as was done by Charters *et al*. (1996) in this study area from 1993-1995. However, in that three-year study, detailed characterisation of only nine out of the several hundred populations they found in 1993-1995 was undertaken due to time constraints.

The question of the varietal origin of the late ferals is important, but would need to be examined in a separate study using a combination of phenotypic screening and molecular techniques. Among available molecular marker technologies, restriction fragment length polymorphisms (RFLPs), amplified fragment length polymorphisms (AFLPs) and simple sequence repeats (SSRs) have been developed and applied in oilseed rape genetics and research. These polymerase chain reaction (pcr)-based marker technologies would need to be targeted at specific populations to return the most information from the costs of consumables and effort. More recently, DNA hybridization-based technologies such as Diversity Arrays Technology (DArT) have been developed for such applications and do not require any DNA sequence information; they also work well in many polyploid species (Raman *et al* 2012). Methods are therefore now available for the screening of large numbers of plants.

2.4.2 Transport routes or farmland?

The report on the first three years (Charters *et al*. 1999) pointed to the concentration of ferals along the main roads. For example, the N-S transport corridor (Table 2.2) contained around one quarter of all the ferals observed in the first two years. Subsequently however, feral populations increased in both transport corridors and

farmland. The farmland contained just more than half the populations but was much greater in area than the corridors. Based on number per unit land area, the corridors always contained more than twice the number of populations per unit land area as the farmland.

The balance of ferals among the three transport corridors also changed over time. The coastal E-W route contained few populations at first then came to support the highest frequency, while the frequency in the N-S route decreased. Road works and the associated import of soil might explain some, but not all such changes. The large accumulations of feral populations in the earlier years along the N-S corridor followed extensive road upgrades and repairs, after which ferals were observed to occupy bare soil along roadsides. These roadworks would have resulted in ingress of soil from outside the area and major soil disturbance in and around the site of the new road as soil was moved around. Claessen *et al.* (2005) used models that demonstrated that more favourable habitats for oilseed rape had high disturbance rates and local seed production that significantly contributed to population dynamics. Crawley and Brown (1995) reported that roadside populations established from seeds imported with topsoil showed dense flowering plant populations in the first spring that the topsoil was put down, followed by very sparse populations in the second year, with extinction by the third growing season. Observations at this site also showed that the very large populations found during and immediately after the road works declined rapidly.

However, no comparable works preceded the major rise in ferals along the coastal E-W route in 1996. A highly disruptive major road upgrade started in this corridor in 2003 and was completed in 2005, but did not result in further major increase in populations

along the E-W coastal route in 2004. More widely, the general increase of feral populations in farmland occurred in the absence of any major disturbance. It appears therefore that the increase in the number of ferals over this 11-year study was mainly determined by factors internal to the region rather than imposed in the form of major construction works.

2.4.3 Are ferals a risk?

Relevance to GM coexistence

The existing calculations of the maximum possible contribution of ferals to impurity in oilseed rape crops were based on the extreme and unlikely condition that all feral seed in a region was harvested as part of the crop - a value which would be much greater than any potential contribution by pollination from ferals in flower. For five regions in Europe, included the data from the Tayside study in the 1990s, the existing feral populations were several orders of magnitude too small to affect an impurity threshold of 0.9% GM in non-GM harvest (Devos *et al*. 2012; Squire *et al*. 2011).

The additional information provided in this study allows confirmation that the increase of ferals in 1996 and 2004 did not change the previous conclusion based on the total area of winter crop in the region, which remained high. Attention is therefore directed to the spring crop and to individual fields that might be at high risk, for example due to being surrounded by ferals.

However, no instance was recorded of multiple, large feral populations in close proximity to a single field. Among large early ferals, only three grew within 10 m of a crop and even if harvested with it (which is highly improbable), their contribution to

seed impurity would have been 10 to 100 times less than the impurity threshold. There were few large populations of late ferals and none of them grew within 10 m of a spring field in flower. At no time in any of the five sample-years therefore were any fields compromised by large accumulations of ferals in the vicinity.

The potential relevance of ferals for coexistence consists therefore only in their capacity to retain traits from crop varieties that have been phased out (Pivard *et al*. 2008; Charters *et al*. 1999) and reintroduce these traits by cross pollination with crop plants or seed transfer to fields in in subsequent years. Any traits entering fields would be managed along with other volunteer oilseed rape, and as argued by Devos *et al*. (2012), such traits would only multiply if they had a selective advantage. If the trait conferred a potential advantage, such as tolerance to a herbicide for example, then control would be possible by other means.

The main source of impurity relevant to the management of coexistence remains primarily through volunteers arising from previous crops, and to a lesser degree to impurity in sown seed and through cross pollination between flowering crops (Squire *et al*. 2012). The few, very large feral populations found here were due to specific circumstances (e.g. soil imported for road works, semi-derelict fields), and were highly visible and could readily be controlled in any scheme for managing coexistence; but even these did not occur close to fields. Therefore, the rise of ferals in the later years of the study was not sufficient to compromise the coexistence threshold for all fields grouped or for any single field.

Relevance to ecological risk assessment of GM crops

The EFSA (2010) guidelines on environmental risk assessment of GM crops in Europe ask whether GM ferals are, or have the potential to be, persistent and invasive, thereby adversely affecting wild relatives, plant communities and their associated food webs. Since GM crops have not been commercialised in most of Europe, EFSA's deliberations are directed mainly at case studies provided by non-GM crops, volunteers and ferals. The possible impact of GM ferals that would arise after commercialisation is then examined in relation to what is known of the local, non-GM comparator.

The value of the present study is that it covers a longer period and a larger spatial area than has been possible in typical short-term projects and therefore presents a unique non-GM comparator. No other such comparator of this spatial scale and duration exists either in Europe or elsewhere.

Previously, risk-assessment of GM oilseed rape has relied heavily on small-scale, ecophysiological studies that had attempted to assess whether sown 'feral' populations survive and increase. For example, in controlled sowings into road verges, field margins and wasteland earlier within the Tayside study area, very few seedlings survived to maturity due to grazing (e.g. by molluscs) and abiotic stress (Charters *et al.* 1999). The same was so of the controlled sowings in different habitats in the UK by Crawley *et al*. (1993). However, the demographic study reported here showed that feral populations increased in number, not just along transport routes but in farmland generally.

The reason for the discrepancy between small-scale studies on risk assessment and the actual rise of ferals here is unclear. Possibly, most seed dropped in ruderal habitats does

not survive to reproduction due to unsuitable local conditions. So it may not be surprising that sowings at only a few sites do not give rise to persisting populations. Alternatively, feral populations might not persist for long at any one site and might persist regionally through redistribution of seed. In this study area of >500 km^2, enough seed in enough locations must have survived or have been redistributed to result in the large increases demonstrated. The fact that feral oilseed rape persisted when the crops, particularly spring varieties, had declined means that the feral oilseed rape would have the potential to harbour traits which could persist and be transferred to other members of the *Brassica* complex at a later time.

However, the area of risk assessment defined in EFSA (2010) that is least researched is that of ecological impact following the establishment of ferals in a region. Several types of impact are possible, but they were found to be absent from semi-natural habitats such as woodland, rough unimproved grazing and wet places. They showed no ability to establish populations in such habitats, possibly due to the absence of competition-free germination sites (Devos *et al*. 2012; Warwick *et al*. 1999; Crawley *et al*. 1993). Possible impacts are therefore confined at present to farmland and other ruderal habitats.

By the end of the survey, however, feral oilseed rape had possibly become the most common crucifer in ruderal habitats. Its rise coincided with a widespread decline in wild crucifers such as *Sinapis arvensis* and *Sisymbrium officinale* that occupy similar habitats (see Chapter 1). Questions arise as to whether feral oilseed rape might be contributing to the decline of these crucifers or might be substituting for them in the ruderal food web.

To date, no one has examined such interactions between feral oilseed rape and wild crucifers.

2.5 Conclusions and next steps

The main finding of the preliminary analysis of this eleven year study is that the number of feral oilseed rape populations increased markedly, while the number and total area of fields cropped with oilseed rape decreased. Ferals maintained similar proportions of winter and spring forms and increased similarly in transport corridors and farmland. The increase of ferals had no further implications for GM coexistence – they were still too few in number – but raise concerns around environmental risk and ecological impact.

The opportunities for interaction with other Crucifers and ruderal communities generally are likely to have increased. Ferals would have come into contact with invertebrate trophic groups living on ruderal Crucifers and might have brought agricultural pests into these communities allowing them to thrive on secondary hosts. The study therefore raises general questions about the role of feralism in the ecology of agricultural regions as well as more specific questions about the importance of ferals in the environmental risk assessment of GM crops. In particular, the severe decline of the late-flowering, spring crops provides a unique scenario in which two types of oilseed rape had been grown for several years in the same landscape and then one of them was withdrawn from production.

Before such questions can be examined, the characteristics of their increase and spread and subsequent impact need to be defined. To approach these issues, persistence and spread are examined in Chapter 3, and impacts on the ruderal community and food web in Chapter 4. Questions include:

1. What are the characteristics of persistence – e.g. for how long do ferals persist in the same locations or habitats from year to year (Chapter 3)?
2. What are the characteristics of spread over time – e.g. do feral populations arise near to and cluster around fields or do they move to occupy new areas (Chapter 3)?
3. Do ferals have particular habitat associations or correlations with landscape features (Chapter 3)?
4. To what extent could feral OSR displace related plants in farmland habitats (Chapter 4)?
5. Do ferals preferentially harbour crop pests compared to ruderal Crucifers (Chapter 4)?
6. Is there an ecological impact of increasing feral OSR populations on associated invertebrate (non-pest) foodwebs (Chapter 4)?

3. Spatial and temporal change in the Tayside long term study area in Scotland

3.1 INTRODUCTION

Chapter 2 showed that feral populations increased and spread, not only in transport corridors, but in farmland that was traversed by minor roads and farm tracks. The increases over time were substantial, especially when compared with the decline in area of crop. The study presents a unique opportunity to examine persistence of populations, the spread of populations into new ground, their possible origin in relation to fields, the connectedness among ferals and fields and their potential ecological effects on other plant species and habitats. The methods and results of previous work on feral demography are first examined to see what can be learned about methods and interpretation.

3.1.1 Persistence in the same place

Persistence in the same place has been observed to occur by reproduction of an existing population, seed drop and by germination from the seedbank laid down at a previous event, such as deposition of seed from farm machinery (Chapter 1). Recurrence in the same place does not by itself confirm persistence, since it could result from re-deposition, but there are studies that have used DNA markers to establish the continuity of a genetic fingerprint over time (Pascher *et al*. 2010; Charters *et al*. 1999).

The length of time that a population persists has been found to be highly variable. In the first three years of this survey, 1993-1995 (Charters *et al*. 1999), most feral populations did not recur, even after seeding heavily. Of the early and late ferals in 1993, only 8% were found at the same locations in 1995. Of the populations in 1995, only 10%

occurred at the site of a previous population in 1993 or 1994. Other demographic studies in northern Europe have shown similar results to the situation in Tayside. For example, work in separate areas of northern Germany by Dietz-Pfeilstetter *et al.* (2006) and Menzel (2006) found that the chances of establishing permanent ruderal populations were very low despite the high reproduction potential of ferals on ruderal sites. The highly transient nature of populations is also shown by the macro-scale demographic work in Canada (Knispel & McLachlan, 2010), the USA (Schafer *et al*. 2011) and New Zealand (Peltzer *et al*. 2008).

In some other instances, however, ferals have been shown to persist for longer. Pascher *et al.* (2010) working in Austria found that feral oilseed rape was able to maintain persistent populations for several years, sometimes retaining older cultivars no longer grown; and Elling *et al.* (2009) in northwest Germany found that oilseed rape persisted at the majority (72%) of sites for at least 2 years, and at some for 4 years. A statistical model used by Pivard *et al*. (2008) in Selommes, France, implied 35-40% of ferals observed probably arose from seedbanks laid down earlier.

The reasons for such discrepancies in persistence time are unclear, but are likely to be due to unidentified varietal characteristics and local conditions. Therefore persistence time cannot be assumed from previous work and needs to be examined in the Tayside study.

3.1.2 Origin and spread

The conclusions of the various demographic studies referred to in Chapter 1 suggest the local and regional contexts have a strong influence on the balance between short and

long-range dispersal. In the 1993-1995 study in Tayside, DNA fingerprinting showed that populations could arise by local dispersal, through seed drop and pod shatter over 1 to 10 m, for instance from a volunteer population nearby. Short-range processes of this type have been found to be dominant where fields are at high density in the landscape but next to roadsides that support feral populations. For example, Pivard *et al*. (2008) estimated that 30-40% of feral populations were due to immigration from an adjacent field in the previous year. In that study the fields were not separated by any barrier from the ferals. In contrast, local dispersal was much weaker in a Canadian study (Knispel & McLachlan, 2010) where neither a field nor field-edge feral populations were likely to give rise to adjacent road-edge populations. A local barrier such as a ditch or wall seemed to be enough to restrict short-range dispersal.

Long range processes appear to be dominant in studies where seed transport across large regions, or even continents, converges into major routes or grain storage depots. The findings around harbours in Japan, where ferals could only have come from seed brought into the country then spilled, showed that local source crops are not essential to establish populations (Kawata *et al*. 2009). In the USA and Canada, ferals occurred along routes that were sometimes distant from fields, and they increased in density towards storage depots and industrial sites (Knispel & McLachlan, 2010; Schafer *et al*. 2011).

The existing studies have tended to concentrate on long-range or short-range dispersal. Questions remain on how both forms of dispersal might operate in Tayside, where the land surface is mainly covered by cropped fields subject to crop rotation (in which

oilseed rape might be grown once every two to five years) and where different fields may be linked by the transfer from farm machinery of soil and seed.

3.1.3 Preferred locations and habitat

The emphasis in previous studies has been on major transport routes or linear features between field and roads (Schafer *et al*. 2011; Knispel & McLachlan, 2010; Pivard *et al*. 2008; Crawley & Brown, 1995). Little demographic work has examined the occurrence of ferals in the general countryside and whether they prefer particular habitats. Even the survey of a 40 km^2 area in France (Pivard *et al*. 2008) included little variation in the way of local habitat.

Only two of the large scale studies - those in Tayside (1993-1995) and in northern Germany (Menzel, 2006) - were conducted across extensive rural areas. Together they established that ferals did not normally occur in woodland or plantation forestry, in wetlands, on sandy areas and dunes or in parkland. (Examination of such areas in the present work, not reported here formally, confirmed this conclusion). Little evidence is available otherwise on whether ferals have habitat-preferences within farmland. Yet any preference for habitat might determine whether and how far ferals become established in rural areas outside transport routes. This is an area of work that requires further study.

3.1.4 Status, main uncertainties and questions

The present evidence is mostly consistent that populations of feral oilseed rape are highly dynamic and show high rates of extinction and unpredictable spatial and temporal distributions (Peltzer *et al*. 2008). However, in all areas examined, sufficient

populations persist for a few years or emerge in new locations to ensure that ferals persist or increase in the landscape as a whole. Such dynamics have been found in widely differing circumstances, from the densely packed fields and wayside ferals of the 40 km^2 region central of France, to very large scale road surveys in the USA and Canada and the randomised large-plot surveys over New Zealand (Knispel & McLachlan, 2010; Peltzer *et al*. 2008; Pivard *et al*. 2008).

Whether feral oilseed rape persists at a landscape scale is no longer the main question. The main uncertainties concern their dynamics and spread in farmland, as distinct from along transport routes. It is unknown from previous studies whether ferals prefer certain farmland habitats in which they could become more established or invasive. Any indications that they might be existing independently of the cropping cycle would have implications for environmental risk assessment. As stated earlier, the Tayside Study area is ideally suited to addressing these issues, given its large size, long duration and (uniquely among all feral studies) the integration of transport corridors and farmland.

The specific aims of Chapter 3 are to:

- estimate the persistence of feral populations over time at the same location versus their occurrence at new locations;
- investigate the manner in which feral oilseed rape spreads over space, for example, by short-range movement around existing feral populations, or by entry to new localities;
- examine whether ferals arise close to current or previous fields;
- determine whether ferals are concentrated in some areas and absent or sparse in others and if so to examine the characteristics of these areas;

- consider the effects of the changes in ferals and fields on potential pollen connectivity with current fields;
- to conclude whether ferals behave differently with respect to the above features in farmland as distinct from in transport corridors;
- finally, to assess whether they deserve further study as an ecological risk in Europe, either as they are, or if they came to contain GM traits.

3.2 MATERIALS AND METHODS

3.2.1 Ground surveys and geographic information system

The methods of conducting the extensive ground surveys in Tayside, UK during 1993, 1994, 1995, 1996 and 2004 were described in Chapter 2. The locations of all ferals and fields were recorded at the time of flowering of the winter and spring crops and transferred to MapInfo v10.5 Professional, a Windows-based mapping and geographic analysis application. The data were layered spatially with reference to digitised Ordnance Survey maps as a base layer, allowing identification of individual fields, roads, tracks, wooded areas and other features. Data for cropped fields and feral populations were entered as separate 'layers'. MapInfo allowed visual representation of the spatial data and analysis of the data by various methods, producing grids and using the embedded SQL (Structured Query Language) query functions.

MapInfo was used to estimate distances between objects, for example between feral populations and between ferals and fields, and to construct polygons and grids over the region. Voronoi polygons (Figure 3.1) are used in the first instance to assess the space around ferals. They were first used in ecological studies by Brown (1965) and Mead (1966).

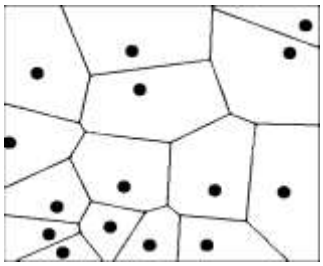


Figure 3.1 Diagram of Voronoi polygons constructed for a set of populations indicated by the dots.

Voronoi tessellation is an "exhaustive partitioning of space in a finite set of non-overlapping continuous regions defined from a finite set of distinct points" (Okabe *et al.* 1992). Each object (for example, a feral oilseed rape locus), is enclosed in a separate polygon. For any set of objects in a two or three dimensional space, a polygonal shape surrounds the object in such a way that approximately any point in the polygon is closer to its object than any other object.

3.2.2 Use of a regular grid

Previous demographic studies have looked at ferals and fields in continuous linear segments (Crawley & Brown, 1995; Pivard *et al.* 2008) or discontinuous random or systematic sampled plots (Schafer *et al*. 2011; Knispel & McLachlan, 2010; Peltzer *et al*. 2008). In order to cover both transport corridors and farmland, a sampling grid was laid over the entire area. Such an approach is applied widely in ecological studies to assess change in occupancy of species, populations or other features over time (Preston, Pearman & Dines, 2002).

In order to assess scales at which ferals occurred over time, grids ranged in size from 10 by 10 m up to a side length of several km. The starting point for each grid was a location in Dundee in the south west corner of the area - grids were then laid out using MapInfo from the starting point to cover the whole of the area. For the subsequent analysis of occupancy by ferals, the following attributes were considered to be essential features of a grid: each square should contain at least one oilseed rape field (defining the potential source of seed) and at least one road running the equivalent of a side length (defining the main potential habitat and a further, secondary source). Estimates based on mean field size and relative oilseed rape land cover (Chapter 2), supported by the analysis using Voronoi polygons, suggested that an area likely to contain on average one or two fields per year would be a square of around 2 x 2 km. A grid of larger size smoothed out some of the variation in altitude and other habitat, whereas a grid of smaller squares, 1 x 1 km, revealed about one third of the squares did not contain a road or a field in any year. Placing a 2 x 2 grid over the region defined 123 squares, all except a few in urban areas containing several fields (though the number varied widely, as explained under Results).

3.2.3 Environmental data

Climatic variation during the during the study period was assessed from certified records of weather data collected at the meteorological station located at the James Hutton Institute to the west of Dundee, at the south west corner of the area. Daily records, summarised and provided by Mark Young as monthly means, are used in the interpretation of trends.

Climatic gradients occur from the south and west of the area of Dundee, and inland to the north and east. Studies in the 1970s by E. L. Birse and colleagues defined the climate for vegetation type and plant growth by three main sets of variables: temperature and dryness (estimated as soil water deficit) which decreases inland mainly with altitude; and oceanicity, which is the dampening effect of the sea on the annual range of temperature and which reduces the number of days of frost nearer the sea (Birse *et al*. 1971; Assessment of Climatic Conditions, 1. Based on Accumulated Temperature and Water Deficit; 2. Based on Exposure and Accumulated Frost; 3. The Bioclimatic Sub-Regions). Actual climatic data were not available at the scale of 2 x 2 km, so grid squares were characterised by altitude, taken as the average of the spot height points in each square, and nearest distance from the centre of the square to the sea, both estimated from the base map.

For roads, the distinction is made between main or A roads, and minor B and C roads and unclassified roads (www.scotland.gov.uk/stats/bulletins/360/00360-57.asp). Each of the three transport corridors identified in in Chapter 2 is centred on an A road. Those running N-S and the coastal E-W roads mostly now have two lanes either side. The inland E-W road has a single lane either side. As indicated above, each 2 x 2 km grid square is traversed by at least one road. In the farmland areas, these are B and C roads, the latter usually narrower than the B road but still wide enough to have left and right sides marked in most instances.

3.2.4 Connectivity over distance

Separation by distance from crops raises questions about cross pollination and gene transfer to ferals. Based on information on cross pollination in the study area and more

widely (Squire *et al*. 2008; Ramsay *et al*. 2003), populations that are very close to fields are likely to experience relatively high levels of cross pollination. In contrast, ferals that may be several kilometres from a field in flower at the same time will be mostly self-pollinated. The change in distribution of fields and ferals over time provides an opportunity to assess whether changes to the degree of cross pollination were likely. Squire *et al*. (2008) and Ramsay *et al*. (2003) show cross pollination is likely to be in the range 0.1 to 1% at a distance of 10 m from a field, 0.01 to 0.1% at 100 m and 0.01 to 0.001% at 1000 m. The change in proportion of ferals at these three distances from fields is estimated as the number of fields and ferals changed over time.

3.2.5 Statistical analysis

Biomathematics and Statistics Scotland (BioSS) were consulted over and advised on methodological and statistical procedures. The statistical program Genstat version 14 (VSN International) was used for all analysis and curve fitting. Primary data are generally presented as untransformed means. After discussion with BioSS, data such as the distance between ferals and between ferals and fields, were log transformed for analysis so that they conformed to a normal distribution. The effects of year, plant type and years of feral occupancy on response variables (areas, distances, characteristics of locations) were tested using ANOVA for unbalanced designs. For example, year was declared as a factor with 5 levels (1993, 1994, 1995, 1996 and 2004) and plant type a factor with 2 levels (early and late flowering).

3.3 RESULTS

3.3.1 Broad changes over time

Aspects of distance and area are examined by ANOVA for unbalanced designs to summarise broad differences between years (factor with up to five levels) and type of feral (factor with two levels, early or late). Two attributes are considered: the area of voronoi polygons constructed around each feral, which is indicative of the space occupied, and the feral-to-field distance which may be indicative of potential connectivity by pollen. Untransformed values are cited below; data were log-transformed for analysis.

For polygon area, highly significant effects as cited below were found for year ($P<0.001$) and type ($P<0.001$). Area was four to five times larger for late ferals than earlies, consistent with the smaller number of lates. The mean area of voronoi polygons around each early feral decreased from 648 ha in 1993 to around a third of this, 213 ha, in 1996. A subsequent smaller decrease at 2004 was not significant. The mean area for late ferals decreased from 2001 ha to just less than half, 952 ha, between 1993 and 1996, and again no further significant difference. The maximum polygon area also showed major change: for earlies, it was 2460 ha in 1993, decreasing to 1940 ha in 1996, then 1070 ha in 2004; corresponding figures for late ferals were 5220 ha in 1993, 3580 ha in 1996 and 3160 ha in 2004. The large mean area of the polygons, typically hundreds to thousands of hectares, is due to the low density of ferals in the landscape.

The mean distance between a feral and the nearest field in the same year did not change systematically over time for early ferals (untransformed mean of 525 m), but increased

very substantially for late ferals from a means of 834 m in 1993 by almost a six-fold increase to 5110 m in 2004 (differences between 1993 and 1996 not significant).

The broad-scale factors that operated to cause these changes in polygon area and distance to fields were the decline in fields and rise in ferals. For late ferals, the very large decline in fields dominated, causing – despite a spread of ferals indicated by the reduction in polygon area – a steep rise in the distance between fields and feral. For early ferals, the changes are more difficult to interpret, but the large rise in number of ferals against only a small fall in field number and area were not accompanied by any temporal trend in distance. It may have been that there were always enough early fields in the landscape for one to be within a few hundred meters of a feral. The implications of these large changes in late ferals for pollen contact between ferals and fields are examined later in this chapter.

The changes in polygon area suggest that ferals did not increase only by very localised spread around existing populations, but that they spread through the region, thereby decreasing mean and maximum polygon size. However, the actual degree of persistence at original sites and the prevalence of localised spread into the immediate neighbourhood remain important factors in any assessment of the mechanism of ferality and its potential ecological risk.

3.3.2 Persistence at locations and short range spread

The degree to which populations remained at the same location from year to year or potentially moved by short-range dispersal is estimated by measuring the distance from a population in one year to those in previous years. Distances are shown for 1996

populations in relation to earlier years by cumulative plots of percentage of ferals on distance (log scale for clarity) in Figure 3.2. The corresponding analysis for 2004 populations (not shown) was similar. Most ferals occurred at distances between 100 m and 1000 m from one in a previous year. A small percentage of populations, that were variable between pairs of years, did occur at shorter distance and are now examined in more detail.

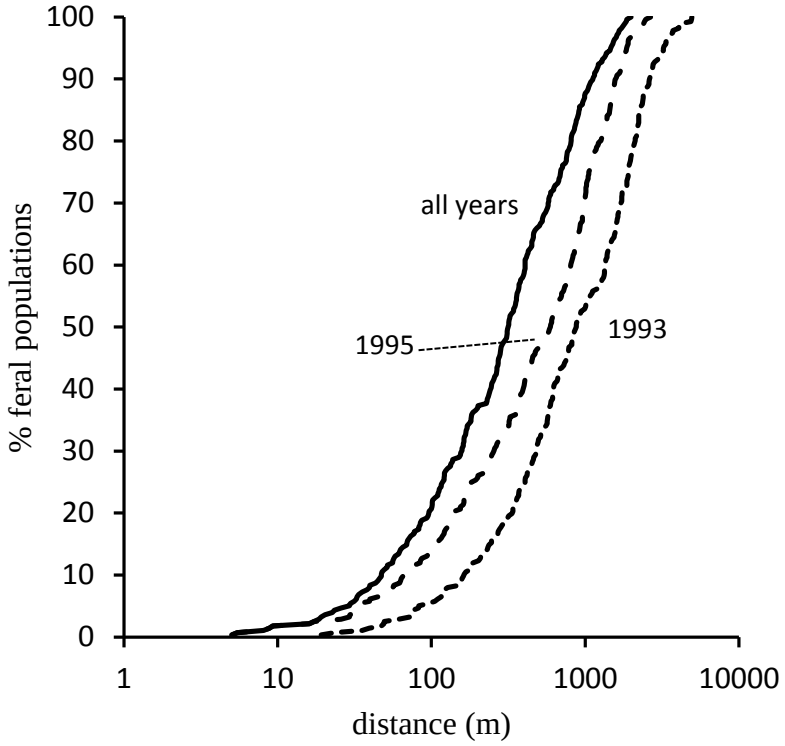


Figure 3.2 Distance between a feral population in 1996 and the nearest population in 1993, 1995 and in any previous year (1993, 1994, 1995)

It was not always possible to determine whether a population in one year was at exactly the same location as one in a previous year. Small populations (up to 10 plants) occurred within a space assumed to have maximum external dimensions of a 10 x 10 m square. However, larger populations sometimes occupied space beyond 10 m, but

rarely more than 20 m. A part of such a population might have remained even though the bulk of it had died. Because of this, values are also cited from Figure 3.2, and similar data for 2004, for a distance of 20 m. Between 1996 and previous individual years, recurrence at one location was restricted to only one or two populations. Of the populations in 1995, as reported by Charters *et al*. (1999), 43% set seed, yet in the next year only 2% of the populations recurred within 10 m and 3% within 20 m. Between 1996 and the nearest one in any of the previous 3 years (early and late ferals not distinguished), recurrence was again 2% within 10 m of a previous one and 4% within 20 m. Between 2004 and any previous population in 1993-1996, the values were little different from those for 1996, 1% at 10 m and 3% at 20 m. By these estimates, more than 95% (the majority) of populations in 1996 and 2004, did not recur at the site of a previous feral. The numbers of recurring populations were too small to compare effects on recurrence of population size or corridors versus farmland. However, of the large populations (100-1000) in 1993, which mainly occurred on the N-S transport route, none persisted to 1996.

Short-range dispersal by seed shatter is sometimes assumed as less than 1 m in the references cited in the Introduction to this Chapter (Elling *et al.* 2009). If this figure is assumed here, then short range dispersal over several years would be not distinguishable from recurrence in Figure 3.2 as described above. But seeds may be propelled over several metres. An extreme value for local dispersal, unaided by vehicles or animals, from a feral population is therefore taken as 10 m. Such dispersal might show itself as an increase in spatial extent of a population or movement in space if a population dies after its seeds have been dispersed. As indicated above, however, few populations

recurred at the same place and none of these showed any temporal pattern of increase in population size (indicative of the need for more space).

The records of Charters *et al*. (1999), coupled with the new data for 1996, suggest that lateral movement by local dispersal occurred for very few populations. Of those present in 1993, less than 1% in 1996 were recorded between 20 m and 50 m (which may indicate lateral spread). The data for 1995 to 1996 are instructive because the number of populations had increased greatly by 1995 from the low values of previous years and increased again in 1996 (Chapter 2). While 43% of the 1995 populations seeded (see above), only 3.6% of these populations were observed in 1996 within 30 m of one in 1995. This percentage includes all those populations that could have either persisted or spread laterally in one pod-shatter event. Between 1996 and 2004 a population could have moved about 80 m, or a distance of 100 m from the centre of a previous 'large' population. For populations in 2004, 16% were within 100 m of one in 1996, but the absence of data for the intervening years means it is impossible to distinguish lateral spread from long-range dispersal into the vicinity.

In total, the data for 1993-1995, together with the new data for 1996, indicate that only a few percent of populations could have arisen by lateral spread due to successive pod shatter over a run of years. Long-range dispersal therefore seems to be the major cause of spread and is now considered for transport corridors and farmland.

3.3.3 Comparison of farmland and transport routes by means of a regular grid

Factors influencing long-range dispersal include the presence and density of fields in the landscape and the number and type of roads. The presence and number of ferals over

time was examined in relation to such factors by means of a regular grid placed over the whole area (see Materials and Methods). Preliminary analysis of grid size showed that persistence over a run of years rarely occurred at small scales (10 x 10 m to 50 x 50 m) which were consistent with the results above in Section 3.3.2. Some persistence over four or five sample years was found in grid squares of 100 x 100m to 200 x 200 m, and it was only when a grid of over 1000 x 1000 m was used that presence or persistence over four or five years was found in a substantial number of squares. As indicated in Materials and Methods, a 2 x 2 km grid was used and each square contained at least one field (with minor exceptions, described below) and was crossed for most of its length by a segment of road. This meant that each square was able to be inspected each year of the surveys for both oilseed rape fields and ferals.

The main transport routes, the N-S, the coastal E-W and the inland E-W, traversed 31 squares compared to 85 squares in farmland. Since the transport routes crossed farmland, each square also contained fields. (Three other squares were neither coastal nor in farmland, but were in urban areas. They contained no fields, but some ferals that were likely to have originated from long-range transport).

The number of years that ferals occurred within a 2 x 2 km square were estimated for the four years 1993-1996 and the five years with the inclusion of 2004. Values for each 2 x 2 km square could be therefore 0, 1, 2, 3, 4 or 5 years. The means of number of years that early and late ferals occurred were compared by ANOVA for unbalanced designs with land type as a factor with two levels (corridor and farmland). The four-year period did not differ qualitatively from the five-year period, results for which are shown in Table 3.1. (To clarify, the values of 3.13 for early ferals in transport routes means that

they occurred on average this number of years out of 5). Transport routes contained a highly significantly greater number of recurrences of both early and late ferals than farmland (Table 3.1). Transport routes were at a significantly lower altitude than farmland, but were at no different distance from the sea. Transport routes and farmland had a similar number of fields per unit area. Values for number of fields shown in Table 3.1 are for both early and late combined, and summed for the years shown. The same results, i.e. no difference between transport routes and farmland, occurs if early and late fields are analysed separately.

Table 3.1 Comparison of mean number of years out of 5 in which ferals occurred in transport routes (n=31) and farmland (n=85) with associated number of fields (early and late combined over the periods shown), altitude and distance from the sea, showing P values (F pr) and variance ratio (vr)

	transport	farmland	F pr	vr
early ferals	3.13	1.92	<0.001	18.1
late ferals	1.55	0.94	0.007	7.55
altitude (m)	85.9	110.7	0.01	6.83
distance (m)	9901	9395	ns	0.19
fields 1993-1996	11.06	9.81	ns	0.69
fields 1993-2004	12.45	11.92	ns	0.1

Of 8 squares in which ferals occurred in all five years, 6 were on transport corridors. Few squares on transport corridors had low occupancy; and no squares had zero occupancy. Only 2 squares had 1 year occupancy which was unlike the situation for farmland that is examined below. The main finding of this analysis was that for a given number of fields in the vicinity, transport routes had a greater occupancy of ferals than the farmland.

Occupancy on transport routes, treated as a factor with 5 levels (1, 2, 3, 4 and 5 years), did not vary significantly with altitude, distance from the sea or mean number of fields per year (early, late or combined). There was no significant relationship between occupancy of early and late ferals across the categories; i.e. a high occupancy of earlies was not associated with a high occupancy of lates.

3.3.4 Variation of feral and field occupancy in farmland areas

Occupancy in the farmland regions ranged from 0 to 5 years. For early ferals, 16 squares had no occupancy, 21 were occupied for 1 year, 18 for 2 years, 16 for 3 years, 12 for 4 years and only 2 squares had ferals present for five years. For late ferals, the most frequent category was no occupancy (36), then 1 year occupancy (29) and 2 year (10) and 3 year (9). Only one square had four year occupancy and no squares were occupied for 5 years.

Occupancy was examined in relation to the number of fields, as an indicator of the intensity of oilseed rape farming, altitude and distance from the sea. The analysis was repeated for both early and late fields that were treated separately and then combined for the years 1993-1996 and 1993-1996 plus 2004. Even though fields declined with time, there were no qualitative and only minor quantitative differences to the outcome using four or all five years. Data are presented as means of the five years.

For early fields, using occupancy category as a factor, with five levels (0, 1, 2, 3 and 4 years; 5 was excluded because there were only two values), ANOVA showed strong effects of number of fields (all fields, F pr, 0.001; vr, 11.97) and distance from the sea (F pr, 0.025; vr, 2.95) but a non-significant relation with altitude (F pr, 0.07, vr 2.26).

The result for fields was similar if winter fields or all of the fields were used. The relation for number of fields is plotted as mean number of fields per year of winter and spring combined in Figure 3.3. Zero and 1 year occupancy occurred with the lowest number of fields, around 1.2 per year. The increase at 2 years was highly significant (<0.001, tested by least significant difference from ANOVA), but the further increase apparent between 2 and 3 years was not significant. There was no further change between 3 and 4 years (the coordinates for 5 years shown for comparison but without SE).

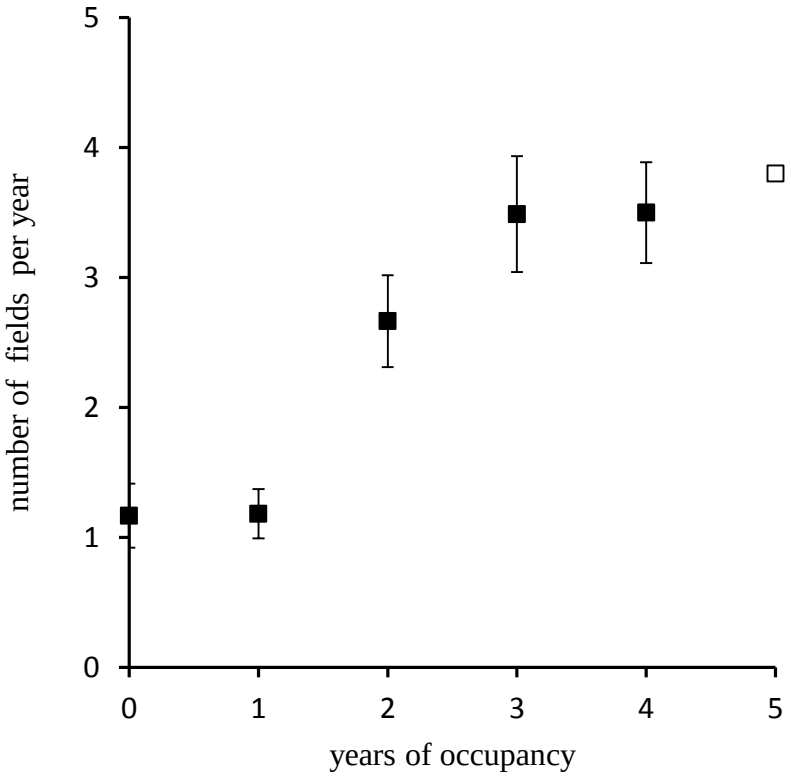


Figure 3.3 Relation in farmland area between number of years (out of 5) in which early ferals were present and number of all fields per year (+ and – SE). The symbol at 5 years without SE is a mean of two

For early ferals, distance from the sea was not significantly different between 0 (mean of 10.4 km), 1 (10.1. km) and 2 year (11.2 km) categories, but declined sharply and

significantly (see above) from these to the 3 (6.5 km) and 4 year (7.33 km), between which it did not differ. Number of winter or all fields was not itself related to either distance from the sea or altitude (regressions not significant). However, areas of zero and 1 year feral occupancy were in squares characterised by a low number of fields and long distance from the sea. These areas were still primarily agricultural in that they were not dominated by forestry; some individual squares had two to three fields per year of oilseed rape but still had no ferals. All had at least one road running through them. Similarly areas of high occupancy (3 and 4 years) had three to four times as many fields and were about half the distance from the sea.

For late ferals, the findings were different. Unlike in the transport routes, the occupancy of late ferals was associated with early occupancy categories (F pr, <0.001; vr, 6.18), increasing from a mean of 0.06 at zero early feral occupancy (one square had a late feral) to 1.4 at 4 year feral occupancy. There was a strong relation between occupancy and number of winter and spring fields combined (F pr, 0.005; vr, 4.08) and number of winter fields (F pr 0.012; vr, 3.46); but there was no significant relation with number of spring fields (vr, 0.77). The relation between categories of occupancy and mean number of all fields was not qualitatively the same as Figure 3.3. A significant difference occurred between the zero (1.66 all fields per year) and all other categories, among which there were no significant differences (mean across categories, 2.94 all fields per year). Unlike for early ferals, there was no relation between occupancy categories and distance from the sea.

3.3.5 Occupancy, population number and population size

Occupancy over 5 sample years has been used to define the areas where ferals occurred. However, occupancy was also a strong determinant of feral population density (Figure

3.4). As occupancy increased, feral population numbers increased, more steeply on transport routes than farmland and more for early than late ferals. The rate of increase was 3.96 populations per occupancy category for earlies on transport routes (slope of the regression), 2.55 for earlies in farmland, 2.19 for lates in transport routes and 1.64 for lates in farmland.

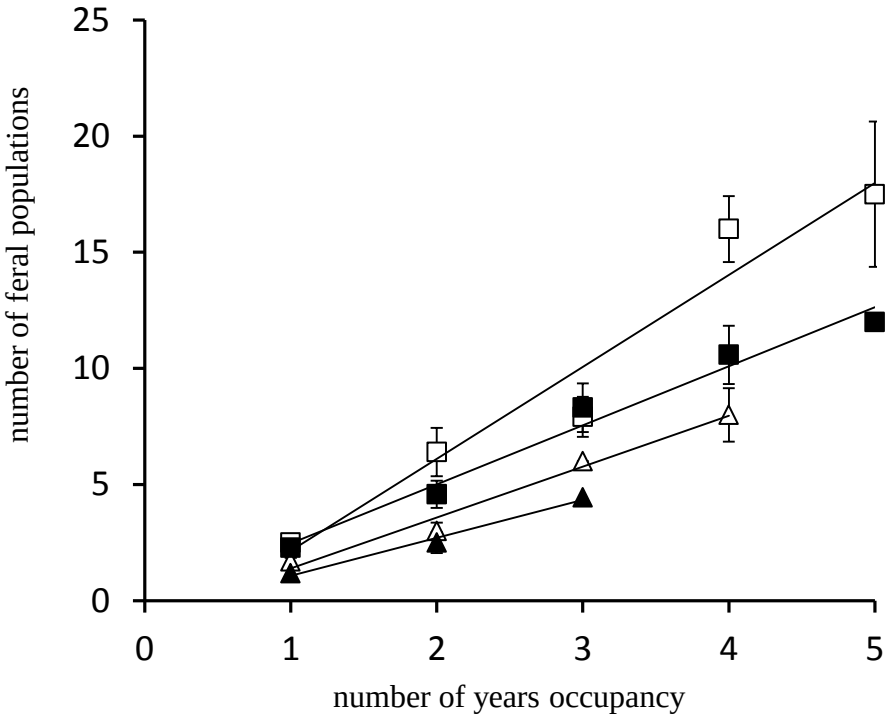


Figure 3.4 Relation between years of feral occupancy and total number of feral populations in (open square) transport route, early ferals; (closed square) farmland, early ferals; (open triangle) transport route, late ferals; (closed triangle) farmland, late ferals (+ and - SE). Lines are linear regressions (see text for slopes; all are highly significant)

The relation between years of occupancy and number of populations in the different size classes (Chapter 2) was broadly similar to that for total populations in Figure 3.4, but

with further shifts towards higher overall density in transport routes than farmland. Large populations in the categories 100-1000 and >1000 plants were more prevalent in transport routes (mean of 1.176 per 2 x 2 km square) than farmland (mean of 0.292). Farmland contained a higher proportion of small populations (58% in those from 1 to 100 plants combined) and fewer (39%) of the large ones. In routes, these large populations were concentrated in the 4 and 5-year occupancy categories, and in some instances (on the N-S route) four such populations were present at some time in the period in a single grid square. In farmland, the fewer large populations were spread across the occupancy categories and no square contained more than 1 large population in the five years. In relation to Figure 3.3, the 1 and 2 year categories contained a similar number to the 3 and 4 year categories.

3.3.6 Distance from ferals to fields and implications for pollen contact

The distance between ferals and nearby fields is relevant for two reasons – fields may be the origin of ferals and potential sources of pollen for cross-pollination. There were examples cited in the earlier work by Charters *et al*. (1999) of ferals appearing within a few metres of a field grown in the previous year. Further examples were noted in the later years. In large parts of the region, fields gave rise to ferals indirectly and results presented earlier in this chapter point to distance from a field not being a useful indicator of origin in most cases. The main transport routes, particular the N-S and coastal E-W, where feral populations are at their highest density, might pass close to fields. However, there is no direct road connection to the fields for most of the length of the routes and so ferals could not originate on these routes by local dispersal of seed from the fields. At the other end of the scale, some farmland areas supported fields year after year, but no or few ferals were recorded in the vicinity (Figure. 3.3). Where fields

are at their highest density in farmland, many ferals are located close to the boundaries of previous fields but there is no means to distinguish origins, since fields have been grown in these areas at high density well before the observations began in 1993.

The large changes over time in fields and ferals, together with large spatial differences between transport routes and farmland and within farmland, had possible implications for pollen contact between ferals and fields. The change over years in distance between flowering ferals and both other flowering ferals and flowering fields was therefore examined to assess possible impacts on cross pollination rates. Data for transport corridors and farmland are combined since pollination may occur freely between these areas.

Feral to feral distance (both in flower) decreased over time as the number of populations increased and spread (Table 3.2). Distances are presented as back-transformed means. For early ferals, the distances in 1995, 1996 and 2004 were all greater than those in previous years but not significantly different from each other. The higher, though non-significant, value for early ferals at 1994 compared to 1993 was due to the uncharacteristically smaller number of early ferals that year. For late ferals, significant change in distance occurred between the first three years and the final two years.

Table 3.2. Mean distance (m) between feral population and the nearest feral in flower in each year with results of ANOVA for unbalanced designs performed on log-transformed data. Values for distance are back-transformed means. Different letters in a column indicate significant difference between means

year	early ferals		late ferals	
1993	614	a	1678	a
1994	576	a	868	a
1995	274	b	1069	a
1996	198	b	434	b
2004	248	b	681	b
F pr	<0.001		0.001	
v. r.	16.45		4.71	

Feral to field distance (Table 3.3) changed over time in a different way to feral to feral distance. For early ferals, the change was not systematic but the distance in 1996 dipped for reasons that are uncertain, but which must have been due to a change in the spatial distribution of fields and ferals. For late ferals, the trend was for a longer distance as the number of fields decreased to almost zero in 2004 but because of large variation the differences were significant only between 2004 and previous years.

Table 3.3. Mean distance (m) between feral populations and the nearest field in flower in each year with results of ANOVA for unbalanced designs performed on log-transformed data. Values for distance are back-transformed means. Different letters in a column indicate significant difference between means

year	early		late	
1993	284	a	598	a
1994	284	a	575	a
1995	281	a	1135	a
1996	165	b	1270	a
2004	296	a	4355	b
F pr	0.002		<0.001	
v.r.	4.24		21.60	

Comparing Table 3.2 and 3.3, at flowering time, the feral populations were closer to a field than to another feral population in the early years. Subsequent changes had very different effects for early and late ferals. With time, the large rise in early feral population number and small fall in winter cropped fields brought the two distances (feral to feral and feral to field) near together. However, the moderate rise in late ferals and massive drop in spring fields caused feral to feral distance to decrease but feral to field distance to increase to over 4 km.

Possible effects of these demographic changes on likely cross-pollination from fields to ferals is summarised in Table 3.4. Numbers of ferals within 10, 100 and 1000 m of fields are listed for all years and both early and late ferals. Only a small proportion of early ferals, mostly <10% are likely to experience the relatively high crossing due to being near a field; the dip in mean distance in 1996 (Table 3.3) was associated with a rise to 16% of populations at this close range. Otherwise populations are likely to experience low cross pollination below 1 in 1000 (0.1%). Late ferals would likely experience even lower cross pollination; the slight rise in 1994 was related to the relatively large proportion of lates than earlies in that year.

Table 3.4 Summary of possible effects of demographic changes in Tables 3.2 and 3.3 on cross pollination from fields to ferals. Cross pollination ranges are given under each distance (see Materials and Methods); n is number of populations, within the stated distance for the year and type, and the adjacent percentages are estimated from the n-value and the total number of populations for the year and type

Year	**Type**	**1-10 m 0.1-1%**		**1-100 m 0.01-0.1%**		**1-1000 m 0.001-0.01%**	
		n		n		n	
1993	**early**	1	(1.8%)	7	(12%)	49	(86%)
1994	**early**	2	(5.4%)	8	(22%)	24	(65%)
1995	**early**	9	(7.1%)	25	(19%)	103	(82%)
1996	**early**	36	(16%)	66	(29%)	195	(85%)
2004	**early**	6	(2.0%)	63	(21%)	262	(86%)
1993	**late**	0	(0%)	2	(10%)	12	(60%)
1994	**late**	3	(7.9%)	5	(13%)	16	(42%)
1995	**late**	0	(0%)	3	(9.1%)	10	(30%)
1996	**late**	0	(0%)	0	(0%)	14	(30%)
2004	**late**	0	(0%)	0	(0%)	3	(55%)

The relation for population size class (Chapter 2) and distance to fields was examined by ANOVA (not shown), but no significant differences were found. Large populations (e.g. 100-1000) were not therefore situated closer to fields than small ones.

3.4 DISCUSSION

The new findings in this study include the first demonstration of systematic increase and spread of feral oilseed rape, major differences between transport corridors and farmland

in the dynamics and abundance of populations and the potential isolation of late-flowering ferals due to the decline to almost nothing in the area sown with late-flowering spring crops.

3.4.1 Recurrence over time and spread to new areas

Persistence of individual populations

Ferals had, by the end of the study, become persistent, having the characteristics of a weed or ruderal that enables it to take advantage of disturbance in a range of environments (Rao 2000). Persistence occurred at a large scale, however. The common pattern over the whole study region was for most feral populations to disappear after one or two years. Whereas Pessel *et al.* (2001) found that feral oilseed rape could persist in the seedbank on road verges in France for at least 8 years, this sort of persistence via the seedbank seemed to be uncommon here.

Populations of weeds and ruderal plants are often subject to large environmental variability that restricts their persistence (Claessen *et al*. 2005). Even though ferals and volunteers may have arisen from the same or similar variety, ferals can potentially behave quite differently from volunteers (Knispel & McLachan 2010; Pivard *et al.* 2008; Claessen *et al.* 2005; Pessel *et al.* 2001). High rates of extinction at a given location contrasts with the longer persistence times of volunteers in fields (Middelhoff 2011; Begg *et al*. 2006; Lutman *et al.* 2003). Experiments, in which seeds were buried in field soil, then unearthed and tested for viability, have shown variable persistence times of an individual seed lot from one to more than 10 years (Begg *et al*. 2006; Hails *et al*. 1997).

The main life cycle features apply to both volunteers and ferals: soil disturbance allows seeds to enter the seedbank by seed rain from parental plants, deposition from machinery or transportation and animal dispersal, such as being eaten and then excreted (Crawley & Brown 2004). Ferals commonly produced seed in Tayside (Charters *et al*. 1999) and re-emergence from seedbanks has been recorded. The main difference between feral habitat and fields probably lies in the pattern of disturbance (less tied to the crop cycle in ferals) and the quality of soil (much poorer and more polluted for ferals than volunteers). Further studies would be required to separate the various effects.

Regional dynamics

On the basis of the estimates over the four years 1993-1996, short-range dispersal mechanisms from feral to feral could account for no more than a few percent of new populations. It was not the case therefore that large populations became established initially in suitable areas from which new populations arose by short range spread. Most ferals became established at new sites where they had not been observed before. Over time, more such sites arose so that a net gain in number of populations occurred despite a high rate of local extinction. This pattern has been found with feral oilseed rape in many parts of the world (Knispel & McLachlan, 2010; Peltzer *et al*. 2008). The principal new finding in this study stemmed from the long time scale over which the work was conducted that enabled a marked increase to be detected and characterised.

Dynamics at a regional scale will depend on the extent of dispersal and proportion of suitable habitat, which in turn affects the likelihood of further dispersal among sites (Alexander *et al*. 2009; Freckleton & Watkinson 2002). At one extreme, patches may become isolated. Thus once a population goes extinct, there is little chance of

recolonization. These ‘remnant populations’ may still persist, for long periods because of the seedbanks (Freckleton & Watkinson 2002). The other extreme comprises ‘patchy populations’ (Harrison & Taylor 1997), where suitable habitat is abundant, dispersal occurs frequently among sites, and local population dynamics largely determine regional dynamics (‘spatially extended populations’, Freckleton & Watkinson 2002). Ferals in the Tayside region appear to conform more to the latter model of spatially extended populations.

Crops as source seed

Most of the increase in number of populations in 1995 and 1996 and the lesser proportionate increase in 2004 (see also Chapter 2) must have arisen from other sources such as seed laid down in a seedbank some years previously, spilled from fields at sowing or harvest, or redistributed during farming operations or transport of seed to the port or handling depots outside the area. It is difficult to distinguish between these potential sources, but direct loss from fields was probably not a major source, although this route was found in some instances.

The situation in Tayside was therefore unlike that in Selommes, France (Pivard *et al*. 2008), where fields and wayside strips of ferals are hardly separate, and seed from fields commonly gives rise to ferals a few metres away. Here, there were many instances where ferals arose independently of a field. They occurred in urban areas and also at high density on the N-S and E-W coastal transport routes which run through farmland but are not generally well connected to fields by smaller roads. Even small roads are usually separated from an adjacent field by a barrier such as a wall or hedge. There were instances also where many fields occurred that did not give rise to ferals within a

distance of at least 1 or 2 km, for example in the areas of relatively low field density at long distance inland (see discussion later). Most ferals in the farmland areas of Tayside likely arose from local fields but after movement of seed on farm machinery or in transport of grain to local stores or main routes out of the region. Ferals on the main transport routes might have originated from a mix of seed from local fields and other farming areas.

3.4.2 Cause and implications of increase during the period

The cause of the increase in ferals is one of the least understood aspects of the study. For the first two years, there was no change in number, but a differnce between proportions of early and late populations. A substantial increase then occurred over the next two years. The growth was greater in transport corridors than in the general farmland and it was accompanied by a proportionately greater increase in small populations. As stated earlier, it was not the case that large populations remained at or near the earlier sites. New large populations appeared through the area.

There were no obvious changes to the main characteristics of varieties grown. For example food quality rapeseed remained the main commodity. Road works occurred on parts of the routes but their effects were transient and the port at Dundee remained one of the sinks for produce. The one systematic change that was noted was in the weather (meteorological records collated by Mark Young at JHI Dundee). Mean temperature increased between 1993 and 2004 by 0.074 degree Celsius per year ($y = 0.074x-140$, where y is annual temperature as the mean of daily minimum and maximum, and x is year; $r2=0.47$, $p=0.013$). There was substantial annual and seasonal variability in this trend but no statistically significant trend for the months in spring and autumn when

feral oilseed rape would normally germinate. Warmer temperature would increase the rate of germination of seed, but high diurnal cycling is also needed in spring to release dormancy (Squire 1999). Modelling germination and life history development based on weather might provide further insight but are outside the scope of this study.

Implications of restricted geneflow and isolation for early and late ferals

Ferals are known to exchange genetic material with each other, with crops and with wild relatives (Pascher *et al*. 2010; Elling *et al*. 2009) and to form hybrids which retain genetic material from more than one parent and on which selection may act. The systematic changes in the number of oilseed rape fields and ferals had potentially important effects on their connectivity by pollen. As the number of ferals increased and spread more widely into the region, the mean distance between them decreased (Table 3.2). For the first two and three years, most ferals were closer to a field than another feral Table 3.3), and the field would have provided most pollen around the feral. Cross pollination would have been very low if the ferals were largely pollen fertile (which is likely). At the third and fourth years, the distances for early ferals were similar to a field or another feral.

The distances for late ferals changed substantially as spring rapeseed fields were phased out. By 2004, ferals were mostly several kilometres from a field and several hundred meters from another feral. At such distances (see Materials and Methods) cross pollination would have been extremely low (Table 3.4), to a degree that the flowering ferals were in effect isolated and would have been entirely self-pollinated. The implications of this isolation are uncertain. Whether these populations would have had the opportunity to evolve genetically would depend on a range of factors, including

further redistribution of spring seed. However, by 2004 the late feral populations became the main source in the region of phenotypes that were spring-germinating and would not require a winter cold period to induce them to flower. Without variety-specific DNA analysis, however, firm conclusions on the genotypes of late ferals are not possible.

3.4.3 Transport routes and farmland

Ferals were shown to have different degrees of recurrence in the two broad habitats of transport corridors and farmland identified in Chapter 2. The much greater degree of recurrence on transport corridors did not happen due to persistence at the same sites but to the appearance of new populations at different points on the roadsides and central reservations, particularly on the N-S and coastal E-W trunk roads. The pattern suggests rapid extinction of populations but generation of new ones in the locality by repeated seed spillage or redistribution of seed due to vehicles. Population density on these routes was unrelated to field density in the vicinity (unlike in farmland), indicating little lateral connection between much of the routes and the surrounding agricultural land. Transport routes supported ferals in more years and in greater number per unit ground area than farmland. This strong influence of grain transport is consistent with what has been found elsewhere (Schafer *et al*. 2011; Knispel & McLachan 2010).

The recurrence in farmland is of greater interest because there is little other information for this habitat. Traffic is expected to be much less in farmland. The recurrence of populations in a locality over time was related to the intensity with which land was cropped with oilseed rape (unlike in transport corridors), but the relation was not straightforward. At one end of the scale, localities that grew oilseed rape at low intensity (1 to 2 fields per year in a 2 x 2 km square, contained ferals either only once or not at

all. Crop seed, sown and harvested, did not give rise to ferals within the 2 km grid square, yet there are no reasons to suppose that some seed was not spilled. These localities were also farther from the sea than other farmland areas. The decreasing effect of oceanicity might have caused, for example, more frost days, but on the N-S corridor, there were many localities that were farther from the sea and more exposed than those in farmland and these localities had high recurrence of populations. Climate itself is therefore unlikely to be the sole factor. Another possibility of influence associated with distance is the direction and frequency of travel: those localities supporting low abundance of fields might be less trafficked at harvest than more coastal areas, simply because the flow would be from inland to coast.

In farmland, the increased recurrence of ferals showed a strong relation to cropping intensity, but it is not clear whether this was causal or not. Seed spillage is expected to be higher where there are more fields, but the type of wayside vegetation and the patterns of local transport might also have differed among farmland areas. Nevertheless, the different behaviour of ferals in corridors and farmland demonstrate that the populations have to a degree arranged themselves in relation to local conditions beyond those just to do with transport. This is further evidence that ferals may be becoming established like weeds and other ruderals and finding preferred sub-habitats.

Large feral populations

The dynamics and spread of large populations (e.g. 100 to 1000 plants) are important because they contain most of the feral individuals and have the potential to affect management. This is not to say that small populations are unimportant – each

population represents what is potentially an event (spillage, seedbank emergence) that introduces new source varieties and around which selection can act. However, large populations were no more persistent than small ones and were no more likely to spread by short-range dispersal mechanisms such as seed shatter.

Large populations therefore appeared and soon after went extinct in much the same way as small ones. The main finding with respect to population size is the greater occurrence along transport routes and their concentration there in areas of high year on year occupancy. There were only five of these localities, but they formed ‘hot spots’ of feral recurrence and abundance. Inspection of the localities, most being on the N-S trunk road, revealed no particular features.

Large populations, as they occurred here, were not considered in Chapter 2 to be a threat to coexistence of GM and conventional crops because they did not occur in large enough numbers close enough to fields for their seeds to mix with harvested seed. They mostly occurred hundreds of metres from fields and were probably subject to relatively little cross pollination from fields. In farmland, they appear not to have had any association with areas of high field density. Spread into the region would seem to occur proportionately more through small than large populations. However, the size of populations seems to have limited significance for future dynamics or management.

3.5 Conclusions

Implications for GM risk assessment

The final aim of this work on demography was to conclude whether ferals might deserve further study as an ecological risk, either as they are, or if they came to contain GM traits.

The study has important lessons for GM risk assessment. Typically, an assessment would enquire if a GM trait would increase the persistence and invasiveness of a feral plant. Experimental work might establish if the GM has an increased fitness, but it will not be until the GM trait becomes feral that its actual persistence and invasiveness can be assessed. Even if the GM feral persisted and spread, there is no way of knowing whether any changes were due to the trait or to some other factors acting on the feral population as a whole. The changes recorded here happened in the absence of any major change in context and of course in the absence of GM traits in the population. The reasons for the increase and spread of ferals over the 11 years in this region are unclear. Factors such as road works appeared to have an influence, but only a temporary one, and the increase still occurred well away from transport routes. If global factors such as rise in temperature can be discounted, many potential influences remain unquantified. Crop varieties change over time but no information is available on how much they differ in life cycle characteristics such as emergence. Similarly, any systematic changes to harvesting machinery or 'leakiness' in transport are unknown.

Management of ferals would also be a daunting prospect. As Knispel & McLachlan (2010) have argued, the ephemeral nature of individual populations, and the more permanent nature of the whole meta-population, makes management very difficult, if not practically impossible. While large, obvious feral populations can be controlled, it is

the many small and medium sized ones that cannot be managed because, even if they are controlled one year, it is highly uncertain where they will arise in any subsequent year. Management by increased cutting or spraying could itself have deleterious ecological impacts (Devos *et al*. 2012).

As in the countries where GM crops are widely grown (Schafer *et al*. 2011), GM ferals would join existing ferals if such crops were commercialised here. There is reason to suppose that GM ferals would occupy the same habitats. In farmland, probably more so than along transport routes, feral populations would come into contact with ruderal crucifers which, as part of the arable farmland flora, are the most declining groups of species in the UK (Preston *et al*. 2002). There is no reason in principle why ferals should have damaging effects on such plants and their food webs but there is very little knowledge of the relative performance of feral oilseed rape and wild crucifers. Comparisons of these plant types and the insects on them are therefore the main subject of Chapter 4. The information gained in Chapters 3 and 4 is then used as a baseline which to assess the ecological impacts of more specific GM traits such as insect resistance in Chapter 5.

4. Comparison of performance and function of feral oilseed rape (*Brassica napus*) and charlock (*Sinapis arvensis*)

4.1 Introduction

The increased prevalence and spread of feral OSR (Chapter 3) has the potential to influence the productivity and function of other plant species. Crucifers are known to be invasive in other regions of the world. For example, Garlic Mustard (*Alliaria petiolata*) was first recorded in the United States in 1868 in New York (Knopf, 2001). Once rare, it has spread rapidly and has recently been found in 38 states. It is especially invasive in forests, where it can become so abundant as to affect native species. Black Mustard (*Brassica nigra*) is found throughout most of North America, except the far north, and is rare in the South Eastern United States (Knopf, 2001).

A newly invading plant has the potential to interact with native species and consequently have additional effects on higher trophic groups (Allendorf and Lundquist 2003; Didham *et al*. 2007). Its impact on such groups would be influenced by the various morphological and biochemical characteristics by which invertebrates sense the quality of habitat provided by the plants. For example, plants in the *Brassicaceae* characteristically produce defence-related glucosinolates of which there are more than 120. The enzyme myrosinase, which is stored in specialized plant cells, converts glucosinolates to toxic isothiocyanates (Hopkins *et al*. 2008). Specialist insects can reduce the toxicity of glucosinolates and their products by excretion, detoxification or behavioural adaptations (Hopkins *et al*. 2008). The unappetising and toxic breakdown products usually deter generalist herbivores from feeding. However, modern oilseed rape cultivars lack both the variety and concentrations of glucosinolates found in wild

Brassica species (Blunt, 2008). Feral oilseed rape might therefore host a different range of invertebrates than its wild relatives and be able to act as a reservoir for insect pests that can then invade cropped fields.

Comparative effects on higher trophic groups are difficult to predict from physiological or biochemical data alone, without corroborative evidence from the field. No study to date has assessed feral oilseed rape to see if it has an ecological impact on other ruderal crucifer species, or the insect communities associated with them. Such analysis is necessary for environmental risk assessment (EFSA 2010) as the information would provide a baseline against which the likely impact of GM feral oilseed rape on naturally occurring plant species and associated insect communities could be assessed.

4.1.1 Wild crucifers and feral oilseed rape in the UK

The first task in choosing a wild comparator was to assess the feral oilseed rape (*Brassica napus*) and cruciferous plants occupying similar habitats.

Table 4.1 shows some of the commonest crucifers found in the UK and is compiled primarily from data in the New Atlas of The British and Irish Flora (2002) and in the UK wide Farm Scale Evaluations (Firbank *et al*. 2003). Included in this table are counts of crucifers obtained from field sites during a survey of arable weed flora, field management and soil characteristics across selected farms in the arable east of Scotland in 2007 (Hawes *et al*. 2010). The surveys used to obtain information for the New Atlas of the British and Irish Flora (2002) occurred from 1930-1969 and were then repeated during 1987-1999. During each survey the UK was divided up into square grids with counts undertaken in each square. The New Atlas bases its distribution and change

characteristics on the existence of a species anywhere in a survey grid square (i.e. inside and outside fields) whereas the two other surveys recorded abundance inside fields only.

Within Table 4.1, species were assigned to one of 4 categories; archaeophytes (naturalised before AD 1500), neophytes (introduced after AD 1500), casuals (constantly reintroduced), or natives (a species which arrived in a study area without intervention by man) (New Atlas of the British and Irish Flora 2002).

Table 4.1 Common UK-wide crucifers taken from the New Atlas of the British and Irish Flora (2002) with numbers of crucifers obtained from a seedbank study during the Farm Scale Evaluations (Debeljak et al. 2008) and from a Scottish Farm Survey (Hawes et al. 2010). Change Index is an index of relative changes in the number of occupied squares of species mapped in the 1962 Atlas and the 2003 Atlas

Latin Name	Common Name	Type	Status Of Crucifer	Change Index	FSE Rank	FSE (n)	Farm Survey Rank	Scottish Farm Survey (n)
Capsella bursa-pastoris	Shepherd's Purse	Annual	Archaeophyte Alien	-1.01	*1*	15159	1	10761
Brassica napus	Oilseed rape	Annual or biennial, rarely perennial herb	Neophyte Alien	+2.88	*2*	8850	2	3288
Sinapis arvensis	Charlock	Annual	Archaeophyte Alien	-1.76	*3*	2235	3	708
Sisymbrium officinale	Hedge Mustard	Annual or Biennial herb	Archaeophyte Alien	-0.21	*4*	853		0
Brassica sp	not identified to species				*5*	285	6	5
Cardamine hirsuta	Hairy bitter-cress	Winter annual	Native	+0.69	*6*	211	4	48
Raphanus raphanistrum	Wild Radish	Annual	Archaeophyte Alien	-1.39	*7*	92		0
Arabidopsis thaliana	Thale Cress	Winter annual	Native	+1.21	*8*	62		0
Barbarea vulgaris	Winter Cress	Biennial or perennial herb	Native	-0.02	*9*	33		0
Sinapis alba	White Mustard	Annual	Archaeophyte Alien	-0.90	*10*	10	5	6
Thalaspi arvense	Field Pennycress	Annual	Archaeophyte Alien	+0.16	*11*	5		0
Brassica rapa	Turnip	Annual Biennial herb	Archaeophyte Alien	+0.74	*11*	5		0
Brassica nigra	Black Mustard	Annual	Native	-0.02	*12*	4		0

Most crucifers in Table 4.1 are annuals, with the exception of winter cress (*Barbarea vulgaris*), which is sometimes biennial or perennial. The column Change Index in Table 4.1 shows that between the two survey periods of 1930-1969 and 1987-1999 there has been a decrease in *Sinapis arvensis* (charlock) but an increase in *Brassica napus* (oilseed rape).

In the UK wide Farm Scale Evaluations, *Capsella bursa-pastoris* was the commonest crucifer, followed by *Brassic*a *napus*, then *Sinapis arvensis*. It is after this point that the two surveys differ slightly: *Sisymbrium officinale* and *Brassica* species (i.e. plants of this genus not identified to species level) were the next most abundant crucifers found during the FSEs, but *Sisymbrium officinale* was not found at all during the Scottish Farm Survey. *Cardamine hirsuta* was ranked at 6 (FSEs) and 4 (Scottish Farm Survey).

The choice of comparator was then made based on the following criteria: (a) similarly common and abundant to feral oilseed rape, (b) likely to be found in most agricultural areas, and (c) similar in life history traits, habit and biomass to feral oilseed rape. Of the commoner species, charlock was chosen as a suitable candidate for the study because it is similar to feral oilseed rape in size and flowering characteristics, unlike the more common crucifer *Capsella bursa-pastoris* (Shepherd's purse), which is usually smaller in size, has white flowers and produces purse shaped seed capsules rather than pods. Charlock, like feral oilseed rape, is also found growing in ruderal areas, field margins, and within crops in the Tayside Study Area. Charlock is highly competitive with cereal crops and can therefore, like volunteer oilseed rape, be a problematic weed that farmers manage with herbicides as well as through the management of rotations within their cropping systems.

4.1.2 Invertebrate species, functional groups and communities

There are likely to be a wide range of invertebrate species and functional groups living on charlock and feral oilseed rape. There are likely to be herbivores (many of which will be crop pests), detritivores, predators and parasitoids (Hawes *et al*. 2008). Ecological studies tend to examine communities in terms of well established, standard metrics such as species richness, diversity indices and multi-variate criteria (Magurran 2004) and these metrics will be used to analyse field records in the first instance. However, assessments of the effects of change on invertebrate groups are increasingly searching for fewer, more specific indicators – sometimes termed focal species or groups - on which risk managers can concentrate effort (EFSA NTO guidelines 2010).

The use of focal species has arisen particularly in studies of transgenic, insect-resistant crops, containing one or more genes for insect resistance and now grown on approximately 60 million hectares globally (James, 2011). As a part of the larger environmental risk assessment process most regulatory authorities require that developers evaluate the potential of the crops to have adverse impacts on organisms not intended to be controlled by the trait, referred to as non-target organisms (NTOs), (EFSA NTO guidelines 2010). The first step in selecting focal species is to define the functional groups (*e.g.*, herbivores, pollinators, natural enemies, decomposers) that would likely be influenced by or come into contact with the GM crop in the environment where it is likely to be grown. The next is to identify (non-target) species within the identified, relevant functional groups. If relevant, endangered species also need to be listed. Accordingly, the invertebrates found on charlock and feral oilseed rape will be examined for their suitability as focal species.

4.1.3 Aims

This chapter aims to address the following objectives:

- To determine the productivity of charlock populations relative to feral oilseed rape and to assess if there is a potential for oilseed rape to replace charlock in ruderal habitats.

- To examine whether feral oilseed rape has the same or a similar role to charlock in supporting invertebrate food webs, and to assess the potential for an ecological impact in terms of change in resource provision for both pest and non-pest invertebrates.

- To assess evidence for regional differences between populations giving rise to different plant and insect community characteristics.

4.2 Materials and Methods

4.2.1 Geographical locations of agricultural areas

Three areas were selected for sampling and information for each area regarding location, height above sea level, soil type and average annual rainfall are provided to give context. Figure 4.1 shows the location of Carmyllie, Colliston and The Carse in relation to each other within the landscape of the north east of Scotland. Carmyllie and Colliston are approximately 6 miles apart (by road). The Carse of Gowrie is approximately 25 miles from Carmyllie, and approximately 29 miles from Colliston. The three areas range in altitude from 142 m to 10 m above sea level.

(i) Carmyllie

Carmyllie is a rural parish in Angus, Scotland (grid reference NO550420), situated on high ground between Arbroath on the coast, and the inland county town of Forfar (Figure 4.2 (a) and (b)). The main settlements in the parish are Redford, Greystone and Milton of Carmyllie. Carmyllie was formerly known for its stone quarries, which for many centuries produced high quality sandstone which was shipped all around the world. It is now mainly agricultural land, and is 142 m above sea level. It lies within the 'farmland-high' area described in Chapter 2.

(ii) Colliston

Colliston is a small village located in Angus, Scotland (grid reference NO605450). It is within an area that is used primarily for agriculture (Figure 4.3 (a) and (b)). It is a roadside village in the East of Angus, lies 4 miles NW of Arbroath, and is 46 m above sea level and in the 'farmland-low' area in Chapter 2. This area is similar in character to Carmyllie although situated at a lower altitude.

iii) The Carse of Gowrie

The Carse of Gowrie (Figure 4.4 (a) and (b)) consists of low-lying country in the southern part of Gowrie, Perthshire, Scotland (grid reference NO242286). It stretches for about 20 miles along the north shore of the Firth of Tay between Perth and Dundee. The area consists of high quality agricultural land and the area is well known for winter wheat and soft fruit such as strawberry and raspberry, having a southerly aspect. The Carse of Gowrie is 10 m above sea level. The nearest accessible and reliable weather station recorded annual rainfall of 710 mm

during 2006, and was obtained at the James Hutton Institute to the west of Dundee at Invergowrie.

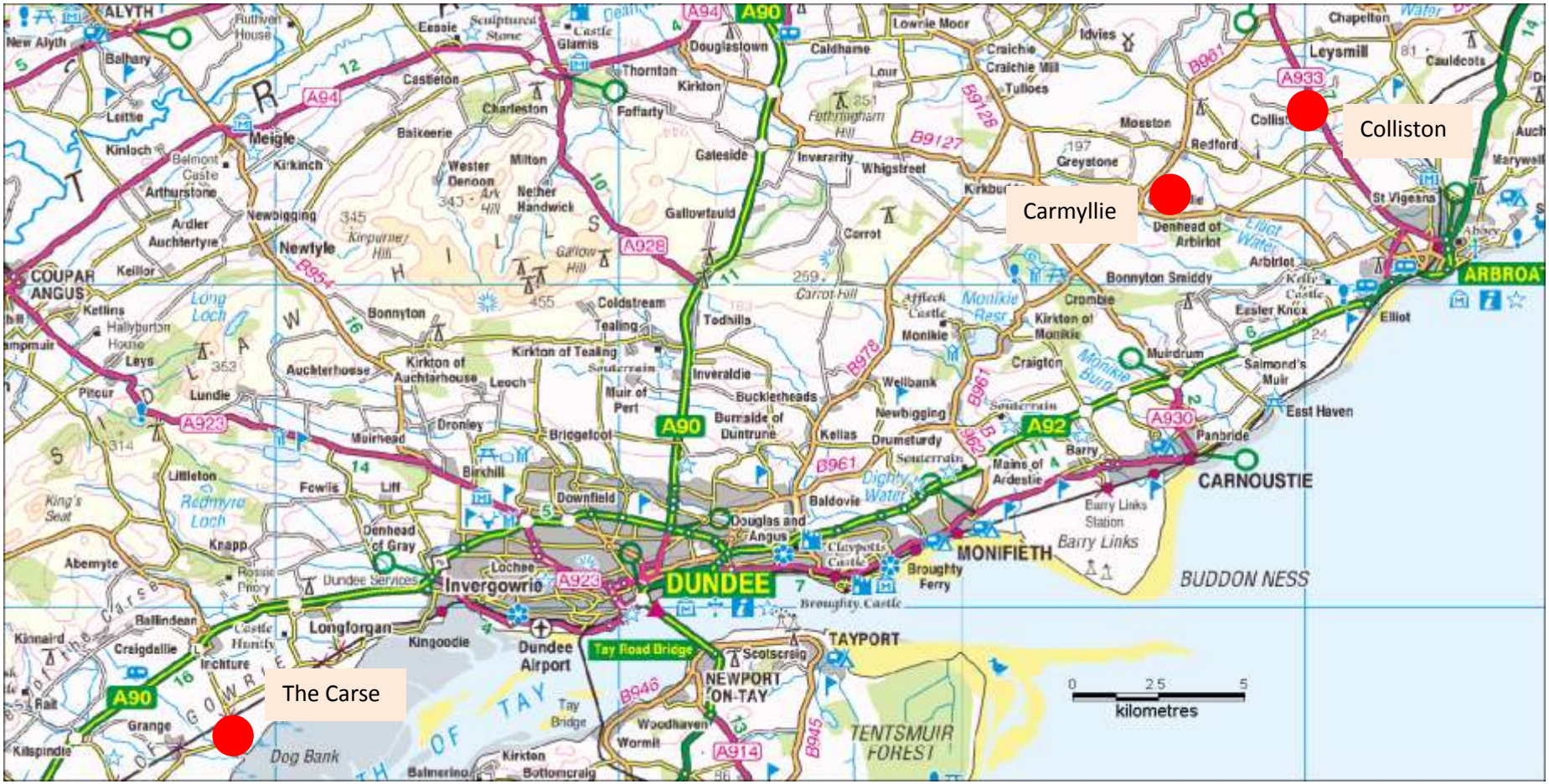


Figure 4.1 Location of regions (red circles) and surrounding areas of The Carse, (left hand side), Carmyllie and Colliston (right side) in relation to Dundee

(i) Carmyllie

Figure 4.2 (a) Generalised location of Carmyllie area showing fields and (b) locations of charlock (blue) and feral oilseed rape (red). Scale bars shown in red

(ii) Colliston

Figure 4.3 (a) Generalised location of Colliston area showing fields and (b) locations of charlock (blue) and feral oilseed rape (red). Scale bars shown in red

(iii) The Carse of Gowrie

Figure 4.4 (a) Generalised location of The Carse area showing fields and (b) locations of charlock (blue) and feral oilseed rape (red). Scale bars shown in red

4.2.2 Harvesting and processing of plants from each of the three areas

During the last week of June and the first week of July 2006, forty plants of feral oilseed rape (38 from The Carse) and forty plants of charlock were sampled within an approximate 4 km^2 area of each geographical region on a single occasion in order to assess the differences between plant types and regions. The sampling of the charlock and feral oilseed rape plants occurred at the same time that sown oilseed rape crops were flowering. Sampling was standardised by collecting plants only on dry, sunny days in late June and July between the hours of 11:00 am and 16:00 pm. This ensured that the maximum number of associated insects would be obtained *in situ*. Plants were selected regardless of size, but had to be flowering (Growth Stage 4, where at least one flower has opened). If there were more than 40 plants within the selected 4 km area, then only the first 40 plants were removed. Plants were recorded by driving and walking along every road in the selected areas to ensure that the sampling would provide comparable data across the three sites. Before any plant was removed each individual had their GPS coordinates taken using a Trimble dDGPS which determined longitude, latitude and altitude. During this process each plant was entirely covered with a 150 x 100 cm polythene bag which was sealed after the plant had been removed from its position. The whole plant was bagged so that all associated insects were also removed. Individual plants were then carefully removed with a trowel to ensure the upper rooting system was taken. Each bag was labelled and plants from each area were taken back to the laboratory and placed in a cold store at 5°C until further processing. In the laboratory, a small cotton wool ball soaked in ethyl acetate was placed in each bag to anaesthetize any insects that were present on the plants to facilitate removal and to ensure that insects did not start to eat each other. The insects were removed using forceps and placed in a 70% ethanol solution in Sterilin 30 ml tubes for storage until identification.

Insects were identified to species (for pest insects) or family (other organisms), and assigned to functional groups as shown in Table 4.2

Table 4.2 Functional groups of insects found on oilseed rape or charlock plants. All insects designated crop pests are herbivores

Insects	Functional Group
Aphids	Crop pest
Earthworm	Detritivore
Beetle larvae	Other
Brassica pod midge	Crop pest
Cabbage stem beetle	Crop pest
Cabbage root fly	Crop pest
Earwig	Detritivore
Flea Beetle	Crop pest
Ants	Natural enemy
Pollen beetle	Crop pest
Lepidopteran larvae	Crop pest
Snail	Herbivore
Springtail	Detritivore
Plant bugs (Auchennorhynca)	Herbivore
Cabbage Seed Weevil	Crop pest
Other weevil	Herbivore
Spiders	Natural enemy
Rove Beetles	Natural enemy
Thrips	Herbivore
True Flies	Other
Parasitic wasps	Natural enemy
Sawfly	Herbivore
Woodlice	Detritivore
Unidentified insects	Other

Because primary production is a key ecosystem process and biomass accumulation can be directly related to plant fitness (reproductive success) the biomass of the plants leaves, stems, pods (if present), and roots were separated and weighed. Fresh weights of these fractions were taken and then the plant material was placed in an oven to dry at 70°C for 48 h, after which the weights of the dry plant material were recorded.

4.2.3 Statistical analysis

All statistical analyses were under taken using the statistical program Genstat version 14 (VSN International). Insect and plant count data was log transformed so that it conformed to a normal distribution.

The effect of plant type and geographic region on response variables (plant biomass and insect number) was tested using ANOVA after confirming that data were normally distributed by using standardized residuals in Genstat. Plant type was declared as a factor with 2 levels (charlock or feral oilseed rape). Region was declared as a factor with 3 levels (Carmyllie, Colliston and The Carse).

Regression analyses were also conducted to compare relations between root and leaf biomass between the different plant types. Here, the mean dry biomass of the leaf material from each plant was the dependent variable and the mean dry root biomass was the independent variable. The factor was plant type (feral oilseed rape or charlock).

Biodiversity (defined here as the number and relative abundance of invertebrates at each of the three sites) was measured as an attribute which had two components – species richness (number of species present) and species evenness (proportional abundance). Several indices and quantitative measures of biodiversity have been developed, with the simplest approach expressing diversity as the number of species in a site or community (species richness). One of the most commonly used indices of diversity in ecology is the Shannon-Wiener Index (H') (Magurran, 2004). This index measures biodiversity, but has the advantage that it takes into account both the number of species and the evenness of the community. The index is increased by having additional unique species or by

having greater species evenness. For this reason species diversity was calculated using the Shannon-Weiner Diversity Index (equation 1) and the Evenness (Equitability) index (equation 2).

$H = -\Sigma Pi \times \ln(Pi)$ _____ Equation 1

where:

H is the Shannon Index

Pi is proportion contribution of each species to the total sampled

ln is the natural log

$E = \frac{H}{Hmax}$ _____ Equation 2

where:

E = Evenness that ranges between 0 and 1, where 0 represents an uneven community dominated by one or a few species and 1 is an even community with the same relative abundance across all species.

H_{max} is the natural log of the total species number.

Insect communities were also described using species accumulation curves (Ugland *et al*. 2003). Several equations relating cumulative species number (*y*) to plant number (*x*) were tested, but the following most consistently gave the best fit to the data

$y = a + bc^x$ _____ Equation 3

where:

y = cumulative species number

a = an asymptote

b = defines the starting value in relation to a

c = defines the shape on the rising part of the curve (the lower the value of c, the steeper the curve at the mid-point)

x = cumulative plant number

The sampling pattern remained the same throughout the three different areas so any difference in the curve parameters is due to shifts in the frequency and abundance of species and not as a result of variation in sampling.

Finally, principal coordinates analysis (PCA) was used to identify differences in the composition of the insect communities between regions and plant types. The Manhattan test of similarity was deemed to be the most suitable for these data (Digby and Kempton, 1987). Similarity is used to describe the association between pairs of samples, and is scored with either a zero or a one.

4.3 Results

4.3.1 Differences in plant biomass between plant type and region

A barchart was plotted of the mean fresh and dry biomass of each type of plant component (Figure 4.5).

Mean fresh leaf weight (biomass) showed no significant differences between the plant type or between area in which they were found (Figure 4.5 (a)), while dry leaf biomass was smaller for oilseed rape but did not differ among areas (Figure 4.5 (b), vr 5.31; P = 0.02). No significant differences were found between plant types for the fresh biomass

of the stem and pods (where present) (Figure 4.5 c) but the fresh stem and pod biomass was greater in Colliston and Carmyllie than in The Carse (Figure 4.5 (d), vr = 5.30; P = 0.006).

Fresh root biomass (Figure 4.5 (e)) was greater for oilseed rape than for charlock plants (vr = 5.91; P = 0.016), and The Carse was found to have the least amount of fresh root between the three areas (vr =10.18; P = <0.001). Dry root biomass (Figure 4.5 (f)) was also greater for oilseed rape than charlock (vr = 5.12; P = 0.025) and area also had a significant effect (vr 4.47; P = 0.012).

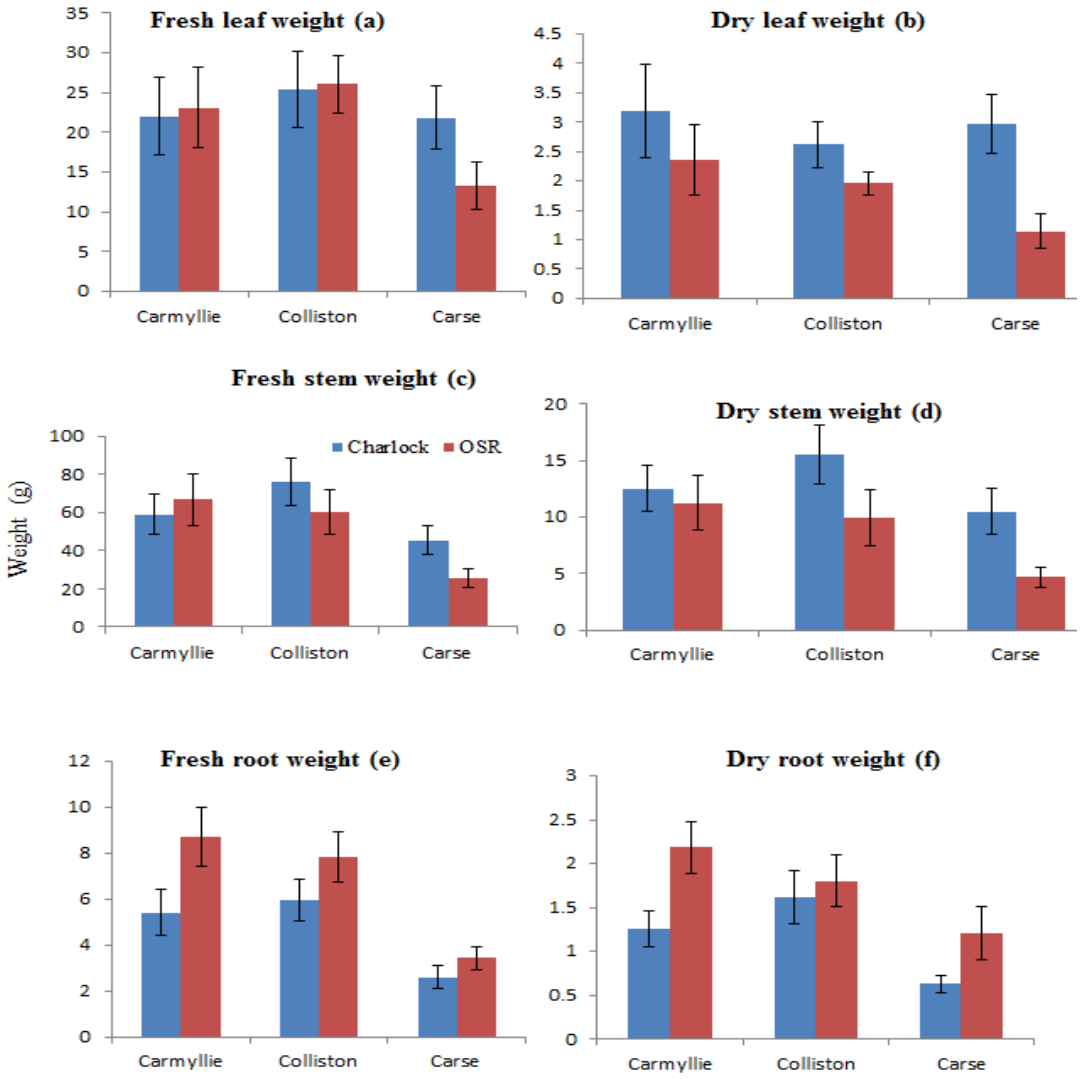
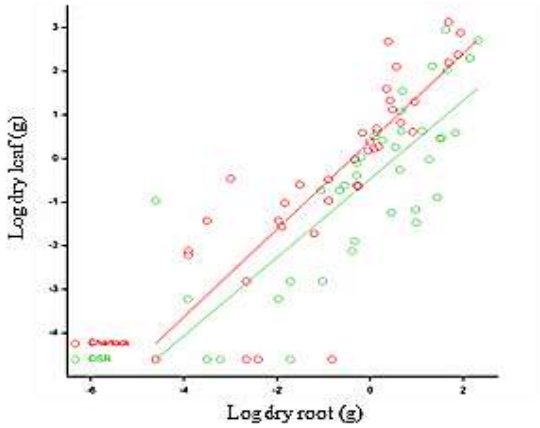


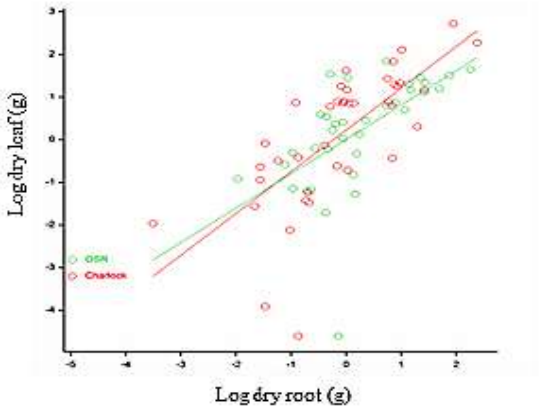
Figure 4.5 Mean ($\pm SE$) fresh and dry biomass of feral oilseed rape and charlock plants from three different areas. (n = 40 for each plant type in Carmyllie, Colliston and The Carse, except n = 38 for feral oilseed rape plants in The Carse)

These differences were investigated further by linear regression of log dry leaf mass on log dry root mass (Figure 4.6).



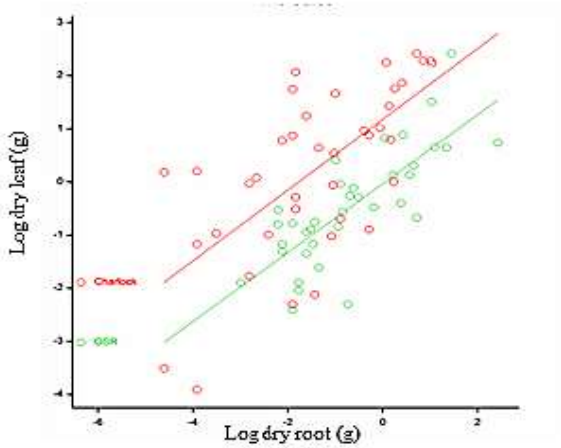
(a) Carmyllie

Charlock: y = 0.394 + 1.006x
Oilseed rape y = 0.469 + 0.898 x
R^2 = 0.62; P = <.001



(b) Colliston

Charlock: y = 0.228 + 0.978x
Oilseed rape y = 0.006 + 0.805x
R^2 = 0.42; P = <.001



(c) The Carse

Charlock: y = 1.177 + 0.666x
Oilseed rape y = -0.035 + 0.649x
R^2 = 0.51; P = <.001

Figure 4.6 Regression analyses of dry leaf and dry root biomass from charlock (red) and oilseed rape plants (green) from (a) Carmyllie, (b) Colliston and (c) The Carse

There is a significant positive relation between root and shoot (leaf and stem) biomass in all three regions. Charlock and oilseed rape were similar at Colliston and Carmyllie, whereas a separation between feral oilseed rape and charlock is evident in The Carse region, indicating a higher ratio of root to shoot dry weight.

4.3.2 Insect abundance on Charlock and oilseed rape plants

Most of the insects that were found on the charlock and oilseed rape plants were herbivores, but natural enemies (spiders and parasitic wasps) were also present (Table 4.2). Other insects such woodlice were associated with a small number of plants, and occurred at very low numbers so were not included in further analyses. The main insects that were found were plotted to compare abundance on charlock plants or oilseed rape plants (Figure 4.7).

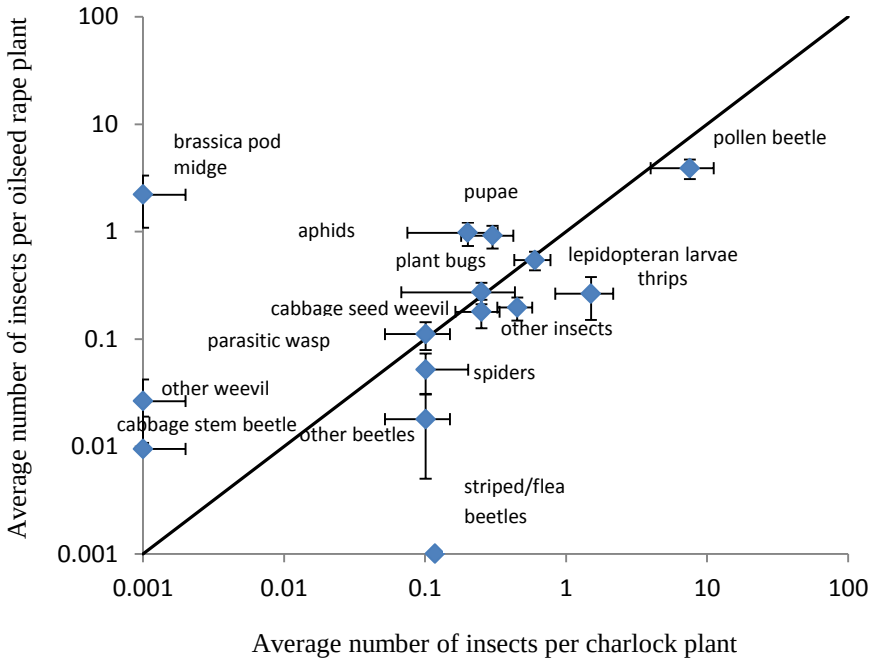


Figure 4.7 Insect abundance on charlock plants (x-axis) and oilseed rape plants (y-axis including error bars. Scaled by n + 0.001 to allow plotting on a log scale. Note – Only main groups of insects found on plants are shown here where insect counts were > 1

From Figure 4.7 it can be seen that 7 insect species or groups occurred at higher abundance on charlock than on feral oilseed rape but two specialist insects - Cabbage stem beetles (*Psylliodes chrysocephala*) and Brassica pod midge larvae (*Dasineura brassicae*) as well as non-specialist weevils appeared only on the feral oilseed rape. Charlock was the only plant type to harbour Striped Flea Beetles (*Phyllotreta nemorum*) and Flea beetles (*Psylliodes chrysocephala*). ANOVA was used to test for significant differences in the numbers of insects between plant types. A summary table is shown below (Table 4.3) showing significance levels for these groups of insects where the numbers were greater than one. Insect species or groups that showed little difference on either of the two plants charlock or oilseed rape included Lepidoptera (crop pests), plant hoppers (herbivores) and parasitic wasps (natural enemies).

Charlock plants harboured greater total numbers of insects, whereas feral oilseed rape harboured a slightly higher total number of taxonomic groups (though the mean number of taxa present per plant was not significantly different between the two plant types, Table 4.3). Overall, insect abundance was much greater in Colliston ($p<0.001$) and taxonomic richness was greater in the Carse ($p<0.001$).

Table 4.3 Insect abundance on feral oilseed rape and charlock plants for those groups that showed a significant difference. Note - not all groups of insects shown +++ very abundant; ++ abundant; + less abundant; 0 not present

Functional Group	Insect group or type	Plant Type		Significance	Area			Significance
		Charlock	OSR	P (SE)	Carmyllie	Colliston	The Carse	P (SE)
Crop pest Herbivore	Aphids	+	+	0.696	++	++	+++	<.001
Crop pest Herbivore	Brassica pod midge	0	+++	0.048	0	++	++	0.041
Crop pest Herbivore	Lepidopteran larvae	++	+	0.263	++	+++	+	0.136
Crop pest Herbivore	Pollen beetle	+++	+	<.001	+	++	++	<.001
Crop pest Herbivore	Striped flea beetle	++	0	0.012	0	0	++	0.001
Crop pest Herbivore	Thrips	+++	+	0.015	++	++	+++	0.020
	Total insects per plant	21.81	9.64	<.001 (2.96)	7.62	24.29	8.06	<.001 (3.63)
	Total Taxa (S)	3.64	3.18	0.08 N/S (0.26)	2.65	3.05	4.54	< .001 (0.31)

4.3.3 Insect diversity, plant type and area

Table 4.4 shows that invertebrate communities on charlock had a lower Shannon index and Evenness than on feral oilseed rape, and this is likely to be due to a more uneven community structure on charlock as the number of species was similar between the plant types (Table 4.4).

Table 4.4 Shannon-Weiner Diversity Index of insects on feral oilseed rape and charlock plants across 3 regions

		Charlock			**OSR**		
		H (Shannon)	**S (Species)**	**E (Evenness)**	**H (Shannon)**	**S (Species)**	**E (Evenness)**
Area 1	Carmyllie	1.281	11	0.534	1.543	12	0.621
Area 2	Colliston	0.967	11	0.403	1.873	12	0.753
Area 3	The Carse	0.800	12	0.321	1.503	12	0.605

Figure 4.8 below, shows species accumulation curves that were plotted for each plant type and area. The start of each line at the left hand side of the graph shows the average number of insect taxa found per plant, reflecting local richness. The asymptote on the graph (far right hand side) represents total richness for that particular plant and region. The slope reflects the diversity and distribution of taxa associated with each plant type and region. The slope of the curve illustrates the level of diversity within each region – the steeper the slope the greater the diversity within a region.

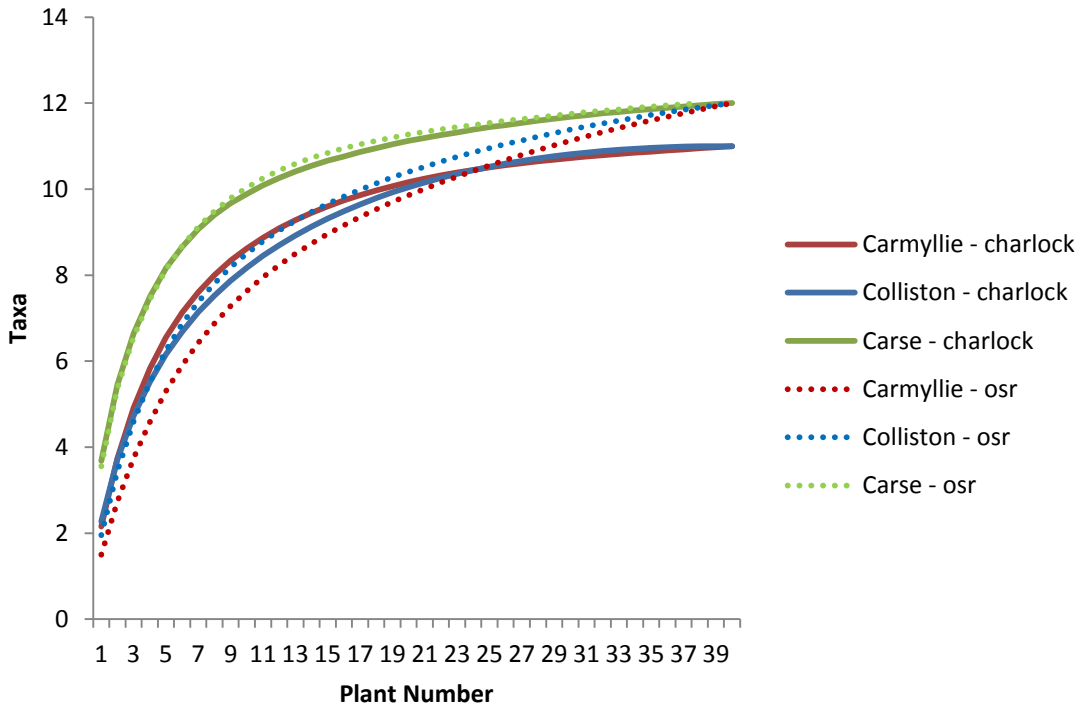


Figure 4.8 Species accumulation curve showing plant type and area

The charlock plants in Colliston and Carmyllie and the feral oilseed rape plants in The Carse appear to have reached the maximum number of species for those regions as the curves seem to be levelling off to an asymptote whereas the other areas do not seem to have reached a maximum number of species. More oilseed rape plants needed to be sampled from Colliston and The Carse in order for the asymptote to be reached, suggesting that total richness in these areas is greater than estimated from these data.

The species abundance curves show that the number of species on each plant type varies only slightly depending upon area and plant type.

Using non-linear regression, standard curves were fitted using the equation $y = a + bc^x$ and the following equations were obtained:

(a) For charlock plants

Carmyllie $10.81 - 9.47 \times 0.86^x$ Variation explained 99.4%
Colliston $11.03 - 9.25 \times 0.88^x$ Variation explained 99.6%
The Carse $11.70 - 8.61 \times 0.85^x$ Variation explained 98.5%

(b) For feral oilseed rape plants

Carmyllie $10.80 - 9.47 \times 0.86^x$ Variation explained 99.4%
Colliston $11.77 - 10.19 \times 0.89^x$ Variation explained 99.0%
The Carse $11.73 - 9.086 \times 0.84^x$ Variation explained 99.1%

4.3.4 Community Composition

Principal coordinate analysis (PCA) was used to summarise these differences in community composition between plant types and regions. Although there were significant differences in abundance between different insect taxa, there was no clear separation in composition of the insect communities by region (Figure 4.9 (a)), or plant type (Figure 4.9 (b)).

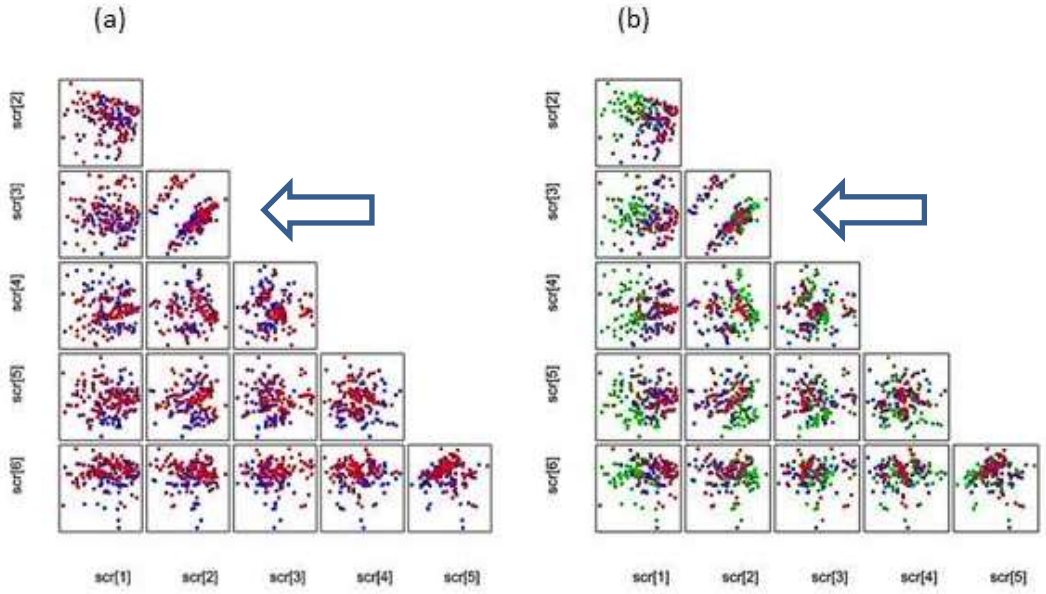


Figure 4.9 (a) and (b) Principal coordinate analysis of insect communities using Manhattan index of similarity between plants showing plant types (a) red = charlock plan, blue oilseed rape; and areas (b) red = Carmyllie; blue Colliston; and green The Carse

Interestingly, there are a group of plants on axis 2 and 3 that appear to be unrelated to either factor. They appear distinct on Figure 4.9 (a) and (b) between scores 2 and score 3 (scr[2] and scr[3]). The smaller group of points contained flies/ other midges (not the Brassica specialist midge), whilst the larger group of points underneath did not contain any flies or other midges. A highly significant difference (P=<.001) was found for the difference between the two groups of points in Figure 4.9 (a) and (b) based on the abundance of flies/midges.

4.4 Discussion

4.4.1 The productivity of charlock populations relative to feral oilseed rape

The mean plant mass and general developmental state of the plants was similar for charlock and feral oilseed rape. Ferals growing in the same or similar habitat were certainly not more productive. There was also no evidence that ferals were competing

strongly with charlock or displacing it. In these three areas, and generally throughout the Tayside region, charlock and feral oilseed rape occupied different patches within the ruderal habitat occurring on waysides and waste ground. On occasions where they were found to cohabit in the same space, the ferals were not smothering the charlock or growing to a higher mass or size. They were therefore similar plant forms occupying similar ground but not in competition.

The study showed however, that there were significant differences in the morphological characteristics of charlock and oilseed rape. Charlock was generally found to have more leaf biomass than feral oilseed rape which may have been because oilseed rape (as a crop) loses most of its leaves by the time it is harvested and this trait would apply to any ferals. The feral oilseed rape in The Carse may have been exposed to more insect herbivore pests than those of Carmyllie or Colliston since insect abundance was significantly greater in this region.

The stem biomass was higher for charlock plants in Colliston and The Carse than those in Carmyllie. This was surprising, because charlock had less leaf biomass than feral oilseed rape in Colliston, but may have been because (generally) the charlock plants were more mature in Colliston, had produced pods thus their leaves had begun to drop leaving the stem and fewer leaves. The plants in this area could have been more mature than the feral oilseed rape (hence the heavier stems), or in an area where the plants were less likely to be disturbed.

Feral oilseed rape had greater quantities of root material in all three areas than charlock, and had a significantly greater root weight in The Carse. This difference in root mass

might be associated with the recent genetic history of modern oilseed varieties which retain some of the characteristics of the swollen upper root of turnip and swede varieties. Also, oilseed rape (as a crop plant) has been bred for its seeds, and to produce the maximum amount of pods that is possible, it would require a large root, both to anchor the plant and to supply nutrients. Charlock on the other hand has not been bred to produce lots of pods and so would require less root material.

Differences between the three areas and between the two plant types may also have been due in part to anthropological processes that were evident at the time of the survey. For example, most of the plants in The Carse were found in an area of recent soil disturbance where soil had been piled up into a bank. Since germinating the plants had then been left undisturbed. Both loosely packed soil and lack of subsequent disturbance may have favoured greater plant size compared to plants along waysides that tended to be managed. Routine maintenance (such as the council cutting back verges along roads or digging up areas to enable improvement of the existing areas), varied depending upon local council policies which would have a major impact on the dynamics of ferals (Squire *et al*. 2011; Pascher 2010; Garnier *et al*. 2008, 2006; Charters *et al.* 1999).

4.4.2 Comparison of feral oilseed rape and charlock in provision for pest and non-pest invertebrates

Feral oilseed rape and charlock, though supporting a similar range of invertebrates differed in several potentially important ways. The number of invertebrates per plant was 4 to 5 times greater on charlock because of much greater numbers of certain species or groups, such as pollen beetle, true flies and thrips, making the community less even than on oilseed rape (Table 4.4). Most invertebrate species or groups recorded,

including predators and parasitoids, were found on both types of plant, but there were certain exceptions. The important crop pests, cabbage root fly and brassica pod midge, were found exclusively on feral oilseed rape (though other pests, pollen beetles and aphids, were found on both). The predator and parasitoids groups were also found on both types of plant.

Such differences are potentially important when assessing the impact of the rise of feral oilseed rape. In a scenario where charlock populations remained stable but feral oilseed rape populations entered or increased in an area, little effect on ruderal food webs would have resulted. The main impact of the rise in ferals would have been through harbouring certain crops pests that were not hosted by charlock. In a scenario where charlock decreased in abundance to be replaced by oilseed rape, the total number of invertebrate species would have changed little, the number of invertebrates would decrease, the highly unneven community on charlock would have become more even, and again the plants together would have hosted more crop pests. There is no firm evidence that the second scenario is currently occurring in agricultural habitats – despite a negative change over time in its presence, charlock is still abundant in and around fields (Table 4.1). The interpretation of these scenarios is based on the consequence of a simple addition or replacement of feral oilseed rape with its associated invertebrates as found here, without any reaction of the invertebrates on either species to the change in balance of host plants. Further consideration would need evidence of why the plant species differed.

Palatability

Differences in insect communities between plant types may be due to differences in plant chemistry. Plant volatiles can play an important role in defending plants against insect attacks by attracting their natural enemies (Wei *et al*. 2007). For example, green leaf volatiles (GLV) and terpenoids emitted from herbivore-damaged plants have been found to be important in host location by parasitic wasps (Wei *et al*. 2007).

One of the characteristics of the *Brassicaceae* is the presence of glucosinolates which occur at varying concentrations in the plant tissue (Gutbrodt *et al*. 2012). Feral oilseed rape may have consisted of different cultivars, especially if a feral population consisted of individuals originating from crops that had been grown for both industrial and food usage. In this scenario glucosinolate levels would vary because oilseed rape for industrial use would not need to contain regulated amounts of glucosinolates, whilst that for food use would be regulated due to toxicity levels. Higher glucosinolate levels could act as an attractant for specialist insect herbivores and a repellent for generalists.

However, the evidence for glucosinolate concentration affecting herbivory in the literature is variable. Some studies have shown that the performance of specialist and generalist herbivores is not explained by glucosinolate profiles. For example, Poelman *et al.* (2008) studied the performances of three Lepidoptera species (two specialists and one generalist), on different cabbage cultivars with differing glucosinolate levels. This study found that within cultivated plants the performance of the three herbivore species did not correlate with the concentrations of total glucosinolates. Gols *et al.* (2008) compared the development of several species of herbivores and endoparasitoids when reared on three *Brassica* populations that differed in their degree of domestication. The

results of this study revealed that glucosinolate levels in leaf tissues varied significantly among the three different plant populations. Much higher concentrations of glucosinolates were found in wild cabbage than in cultivated and feral populations. Both Poelman *et al.* (2008) and Gols *et al.* (2008) were able to show that glucosinolate levels in plants that had been attacked by herbivores increased.

Resource availability and apparency

There are many other factors which have the potential to affect the attractiveness of each type of plant to predation by insects. Firstly, for example, diet breadth increases when hosts are less predictable (stable) and narrows to monophagy when hosts are abundant and numerically stable from year to year (Chew and Courtney 1991). Secondly, infestation levels have been shown to rise when host populations are stable. Predictable and apparent hosts accumulate greater herbivore loads, consistent with predictions and findings for apparent plants (Chew and Courtney 1991). Both charlock and feral oilseed rape plants in The Carse were apparent and stable for several months which could have explained higher numbers of insects found on the plants in The Carse which may have been due to the undisturbed bank that was created at the time of the survey.

Both plant chemistry and apparency have differential effects on insect taxa according to their degree of specialism (Gutbrodt *et al*. 2012). Overall, more specialist insect pests (which, if they occurred within an oilseed rape crop would be sprayed against), were found on oilseed rape plants. Generalist invertebrates (other weevils, woodlice etc.) or non-pests were found more predominantly on charlock plants. The only exception to this was pollen beetles – a specialist insect that occurred at high abundance on both

plant types. All of the herbivorous invertebrates associated with the feral oilseed rape, with the exception of some of the aphids, were specialist insects (broadly monophagous and oligophagous) (Bergon *et al*. 1996), whilst insects associated with charlock were a mixture of generalists (polyphagous) and specialists. There is therefore the potential for oilseed rape ferals to act as a reservoir of pests that can then recolonize cropped oilseed rape fields. The ferals would be able to harbour pests when the crop is no longer available, such as after harvest. The pests could then return to the crop after a new crop has been sown.

Focal species for risk assessment

A method has been proposed for a first prioritisation of focal species that is based on ecological criteria among which the frequency of occurrence is considered a key parameter (Andrade *et al.* 2012; EFSA NTO guidelines 2010). Other criteria include species' exposure to the GM plant, abundance, feeding habits, and likely sensitivity of the invertebrate to the trait. Scientific opinion (EFSA NTO guidelines 2010) requires that at least one focal species is tested per relevant functional group, and that focal species are chosen for the various environments in which the GM crop will be grown.

The studies at the three sites show that several species occurred at a high frequency and abundance and were either characteristic of both species or restricted to feral oilseed rape. There are several potential options for focal species, therefore, but the choice would depend on the nature of the GM crop in question. The data will therefore be used in Chapter 5 to illustrate the choice of focal species under specified scenarios.

4.4.3 Assessing the evidence for geographical separation between populations giving rise to different plant and insect community characteristics

In comparisons of the type reported here, and in risk assessment more generally, a balance has to be achieved between sampling effort to obtain the number of species (species richness) in a designated area and replication of sampling across regions or a biophysical gradient (EFSA 2010).

Sampling within areas

Observed species richness within habitats (alpha diversity) is dependent on sample size, and large samples are often required in order to get a true reflection of species present (Cowell *et al.* 2004). Species accumulation curves can be used to provide an estimate of the link between species number and sample size for a particular region or habitat.

Differences in the richness and relative abundances of species in the sampled communities underlie the differences in the shape of the species abundance curves that were obtained (Figure 4.8). Because all communities contain a finite number of species, the curves would have eventually reached an asymptote at the actual community richness (Hughes *et al*. 2001). The species abundance curves contain information about how well the communities have been sampled and show the sampling of 40 plants per area appeared enough to capture most of the species or functional groups, particularly on charlock. The steepest accumulation occurred around 5-8 plants (Figure 4.8). The curves for feral oilseed rape appeared to be increasing more steeply over the upper range of plants, indicating more invertebrate species or groups would still be found if more plants were sampled. The curves for oilseed rape might have differed due to variation in palatability, resource availability or apparency as indicated earlier.

Differences between areas

The data presented here have shown that there are regional differences between feral oilseed rape and charlock and in the insect communities they supported. Each area had some characteristics not evident in the other areas, but Colliston and Carmyllie were similar in plant characteristics and despite the larger numbers of invertebrates at Carmyllie, the invertebrate communities at these locations were similar in diversity and evenness indices. The Carse differed from the other two areas in aspects of both plants and invertebrates. The trend towards relatively greater root mass and smaller leaf mass in feral oilseed rape was most pronounced in The Carse and the insect communities were more uneven in The Carse, especially on charlock. Also The Carse had the most species per plant and when accumulated across plants (Figure. 4.8).

Sites with high species evenness, such as Carmyllie and Colliston here, generally have a high proportion of species with a similar propensity for being on sampled plants. In contrast, sites such as The Carse have more rare species that have a low propensity for being caught. There is considerable discussion in the literature on the importance of rarity in understanding community assemblages (Thompson *et al*. 2003). Rare has been defined as species that are: (i) broad ranging but generally sparsely distributed; (ii) locally dense but with a very restricted range; (Main 1982). It is unclear from the evidence available, which of these acted to distinguish the community in The Carse.

However, the condition "locally dense but with a restricted range" appeared to determine the presence of the two pests that occurred only on oilseed rape. Brassica pod midge appeared at high density in Colliston and The Carse but not Carmyllie, while cabbage root fly occurred again at high density but only at Carmyllie. The use of three

areas rather than any one therefore strengthened the conclusion that only feral oilseed rape supported these specific crop pests.

The causal links between area, plants and invertebrates are difficult to define since they depend on many factors. Carmyllie and Colliston were broadly similar in topography, and were closest to each other in geographical terms (6 miles). The Carse of Gowrie was the furthest away (up to 29 miles) Carmyllie was at the greatest height above sea level (142 m) and was quite exposed, with fewer boundaries such as hedges or trees to provide protection from the cold winds that blow across the land from the North Sea. In contrast, The Carse was lower in altitude, and therefore slightly warmer, and had a sheltered southerly aspect which may in part explain why more insects were found in that area. Alford *et al*. (2011), for example, showed that movement of the aphid *Myzus persicae* in Europe became depressed after low temperature acclimation but increased during high temperature. On the other hand, Robinson *et al*. (2012) showed that extreme weather patterns in a temperate region of the USA (drought and low rainfall) had a negative impact on butterflies. The dry conditions caused by lack of rain caused plants to grow at a slower rate that affected their ability to be a suitable food source for the butterfly.

Alternatively, the plants within The Carse might have had plant tissues that were more palatable or more apparent (Chew and Courtney 1991).

Any future study of a similar nature would benefit from collection of quantitative data on geophysical characteristics, such as wind speed and direction, temperature, altitudes of each plant, and habitat connectivity. Soil type could also be of importance as a

healthy soil should produce a healthy plant with a higher and better quality biomass that may encourage insect herbivores (and their predators) to feed on the plants. If the soil had more or less of a particular type of nutrient this may make the plants more or less palatable to herbivores, thereby altering the herbivore load.

4.4.4 Further work

Survey data such as that presented here are valuable in assessing real patterns in distribution and abundance of organisms in the environment. However, there are limitations of this kind of data associated with the lack of control of extraneous variables, including microclimatic conditions, soil type, aspect and so on. Here, the main confounding variable was the co-variation of plant growth stage with region. This resulted in some difficulty comparing the relative differences between plant types across regions. This might be overcome by sampling plant and insects at a number of time points through the growing season so that a population trend could be determined instead of relying on a single large sample.

Alternatively, or additionally, glasshouse experiments could be used to control for all confounding variables and isolate the effect of each experimental variable separately. For example, insect behaviour could have been studied under controlled conditions in the laboratory where factors such as the temperature and lighting received could be controlled. An experiment might be devised where insects are placed into a sealed plant growth chamber containing either an oilseed rape or charlock plant to see what the preference would be for a food source. This could be repeated a number of times, using different insects at differing growth stages of the plant and insect. However, glasshouses produce untypical behaviour and dynamics in both plants and insects. For example, Birch *et al*. (2007) found that in a tiered sequence of experiments from growth room,

through glasshouse to field, results in the more tightly controlled conditions were poor predictors of field performance.

Another way to assess insect behaviour is by using a Y-tube olfactometer to ascertain odour preferences between oilseed rape and charlock plants. Several experiments could be set up where air is drawn from the two arms of the Y-tube down the stem and out, so that an insect introduced into the stem of the Y-tube encounters air streams from two sources,. For example one arm might blow in fresh air, while in the other, air passed over a piece of charlock. When the insect arrives at the fork it can then make a decision whether to turn left or right. Over a number of such trials its attraction to charlock plants can be assessed. The same could be done for oilseed rape or a choice can be offered between oilseed rape and charlock.

More research is also needed to determine the precise mechanisms for the differences found between the plant types and the insect communities associated with them. A controlled insect choice experiment where oilseed rape and charlock plants were offered to specialist and generalist invertebrates would show more conclusively which plant type was preferred. A factorial experiment would be set up with individual, caged oilseed rape and charlock plants exposed to a) a specialist species, b) a generalist species, and c) no insects. Plant growth, insect growth rate and population growth would be measured on all plants and all plant material would be analysed for glucosinolate content and plant nutrient concentrations such as amino acids and nitrogen. This would allow the composite hypothesis “that plant type and plant chemistry has no effect on the abundance and fitness of specialist and generalist herbivores” to be statistically tested.

Finally, in relation to risk assessment, the data here can be further analysed to make a preliminary judgement of whether the replacement of charlock by oilseed rape is likely to have an ecological impact on the arable foodweb. This could be done hypothetically, by sequential replacement of charlock with oilseed rape as assessing insect richness, diversity and abundance under each new scenario. Species abundance curves could be constructed for each replacement scenario to generate a hypothesis for likely risk. Using data presented here, however, there would be little difference in species richness in a new oilseed rape dominated community, but there *would* be a reduction in overall abundance of insects present.

4.5 Conclusions

Feral oilseed rape was found in comparable ruderal locations to charlock, but in most instances occupied separate patches of land. The two species accrued a similar total; mass, but differed slightly in the allocation of dry matter between roots, stems and leaves.

Despite the differences found between locations, the results indicate that the two species harboured similar above-ground invertebrates, although those found on feral oilseed rape included two specialist pests. If charlock was replaced by feral oilseed rape in ruderal habitats, the data suggest there would be (a) a decline in the abundance of insects, as charlock was shown to support a higher number of insects on each plant than feral oilseed rape, (b) little impact on insect species richness although composition might shift towards more specialist insects and (c) a greater evenness of the invertebrate community. The reduced abundance of generalists and natural enemies on feral oilseed rape and increased overall abundance of specialists might have wider implications for

the crop pest dynamics in arable systems. The data presented here also suggests that feral oilseed rape could act as a reservoir for pests that could recolonize cropped oilseed fields and would be able to survive when the crops are not available.

5. Ferality in oilseed rape and its relevance to Environmental Risk Assessment

5.1 New information provided by the Tayside Study

The main findings of the 11 year feral oilseed rape survey were that, despite declining areas of crop, the feral populations had increased in number and had spread throughout the >500 km^2 of the study area. Ferals had not colonised perennial or semi-natural vegetation, and were not as numerous as volunteers in fields (Squire *et al*. 2012; Pekrun *et al*. 2006; Gruber *et al*. 2004; Lutman *et al*. 1993). At present population densities, it would not be necessary to manage them as part of any strategy to achieve GM coexistence (Chapter 2). However, some of the first evidence was obtained of ecological roles of feral oilseed rape.

The main potential contribution of ferals is usually assumed to be the dispersal, persistence and spread of traits into in non-agricultural land (for a general discussion, see Raybould *et al.* 2012). However, their capacity to affect ecological processes in agricultural land is important given the ongoing threats to farmland biodiversity (see Chapter 1) and the ability of ferals to retain GM traits. While populations remain in the same location for several years in some environments (Pascher *et al.* 2010; Pivard *et al*. 2008), most populations in Tayside were short-lived. The population as a whole appeared to exist and expand by exploiting opportunities to emerge and reproduce in new locations. Among further new findings were that feral populations increased not only or mainly along transport corridors, as found by several previous investigators (but also in farmland served only by minor roads and tracks (Chapters 2, 3); and that a set of populations, characterised by a distinct spring-germinating

phenology, flowered in the effective absence of pollen-contact with crops, partly due to the great reduction of late-flowering spring crops.

The first ecological impacts of feral oilseed rape were examined by comparison with the growth of the common ruderal plant charlock and the invertebrates it hosted. Ferals had similar growth to the charlock, except in small differences of dry mass allocation between roots (higher in ferals) and leaves (lower in ferals), and there were no indications they were supplanting charlock in ruderal environments (Chapter 4). Ferals supported a similar set of above-ground invertebrates to those on charlock, but their invertebrate community differed in being less abundant, more even, and having slightly higher species richness and including certain specialist crop pests not found on charlock (Chapter 4).

In total these are substantial changes that merit a re-assessment of feral oilseed rape as an invasive plant (5.2 below) and of its role in the environmental risk assessment of GM crops (5.3).

5.2 Status of feral oilseed rape as an invasive plant

Very few other ruderal plants and in-field weeds have been subjected to the level of interest shown in feral oilseed rape. From the first studies in Tayside commissioned in 1992-1993 (Charters *et al*. 1999), the emphasis has been on the environmental, and then economic (Messean *et al*. 2009), risks of ferals in the event that GM oilseed rape would be grown commercially. Recent studies on feral oilseed rape in Canada (Knispel & McLachlan 2010), the USA (Schafer *et al*. 2011) and Japan (Kawata *et al*. 2009) have continued that line of investigation but with actual GM ferals that arose from commercial GM crops.

The Tayside Study has contributed to the scientific study of ferality, weediness and invasiveness, which has a long history in the UK (Leigh and Johnston 1994; Salisbury 1961). The scarcity of records in the weed flora for *Brassica napus* or *B. rapa* suggests that no volunteers survived from the minor use of 'rapeseed' since the 16th century. Most of the present volunteers and ferals appear to have arisen very recently following the expansion of cropped area in the late 1970s and 1980s (Chapter 1). What is perhaps surprising is how quickly they have become established, particularly in the weed seedbank.

How does feral oilseed rape compare with other invasive plants? Its properties are compared to those of some other invasive herbs in terms of the likely effects on animal or human health (toxicity), the environment (competition and difficulty in removing plants), impacts on agriculture, and finally, impact on other ruderal plants in the same locality (Table 5.1). Oilseed rape is less 'harmful' in most respects than the three comparators - garlic mustard, creeping thistle and creeping buttercup. It has only become economically important as a weed in arable fields. Perhaps the most damaging of the other plants is creeping thistle or Canada thistle (*Cirsium arvense*), which has been described as one of the 'worst plants world-wide' (Guggisberg *et al*. 2012), threatening the native flora and responsible for major agricultural losses. Other, well known invasive perennials include Rhododendron (*Rhododendron ponticum*), that was introduced into the British Isles in 1763, and Bracken (*Pteridium aquilinum*) a noxious weed that was originally a lowland and woodland plant, but now also colonises upland areas. Among the invasive plants mentioned above, feral oilseed rape is of a relatively minor concern.

The life cycle of a plant has a strong effect on its invasiveness. Invasive plants are commonly perennial plants, like the creeping thistle and creeping buttercup, and have the ability to move from germination-sites into perennial vegetation. However, some invasives, such as *Alliaria petiolata* are biennial, and spread by taking advantage of germination sites in those parts of the world where they have become invasive. Feral oilseed rape is mostly a spring annual germinating in spring or a winter annual germinating in autumn. However, a few individuals have been found to survive into a third summer in Tayside, following cutting and re-growth from the cut stumps (written records of the Tayside Study 1993-1995; G. R. Squire, personal communication). Whether perenniality would become more common in feral oilseed rape in uncertain at present.

Table 5.1 Impacts of some invasive plants upon human and animal health, the environment, agriculture and other ruderal plants. X, minimal effect; XX moderate effect; XXX high effect. (Based on Leigh and Johnston 1994).

Invasive plant	Impact on humans	Impact on environment	Impact on agriculture	Impact on ruderals	Life form	Geographic distribution	Invaded habitats	Ecology and control
(Brassicaceae) Oilseed rape (*Brassica napus*)	X	X	XX	X	Annual, biennial	Europe Australasia North/South America Africa Asia	Grasslands (need to state where)	Mostly annual and not persistent at high abundance, except within fields. Large seedbank in fields. Can be cut down manually or killed by herbicides
(Brassicaceae) Garlic mustard (*Alliaria petiolata*)	X	XXX	X	XXX	Biennial	Europe Australasia North/South America Africa Asia	Riparian habitats Forests Open Woods Grassland	Quickly dominates understory and eliminates native species Control difficult once established Plants need to be removed over several years Effective herbicide available
(Asteraceae) Creeping thistle (*Cirsium arvense*)	X	XXX	XXX	XX	Perennial	Europe Australasia North/South America Africa Asia	Forests Grasslands Riparian habitats Lakeshores/marshes Dunes	Crowds out native vegetation Regenerates from root cuttings Establishment depends on disturbance Spot mow to weaken plants Herbicides
(Ranunculaceae) Creeping buttercup *(Ranunculus repens)*	X	XX	XXX	X	Perennial	Europe Australasia North/South America Africa Asia	Forests Grasslands Riparian habitats Freshwater wetlands	Seedlings can establish on bare ground Plant has large seedbank Invades natural plant communites Can remove roots manually Effective herbicide

The continued presence of oilseed rape as a volunteer and feral will depend as much on the conditions in field and waysides as on the plant itself. Over the history of agriculture in Scotland, few weeds have maintained their status over long periods without some change in environment or agricultural practice that has given them opportunity.

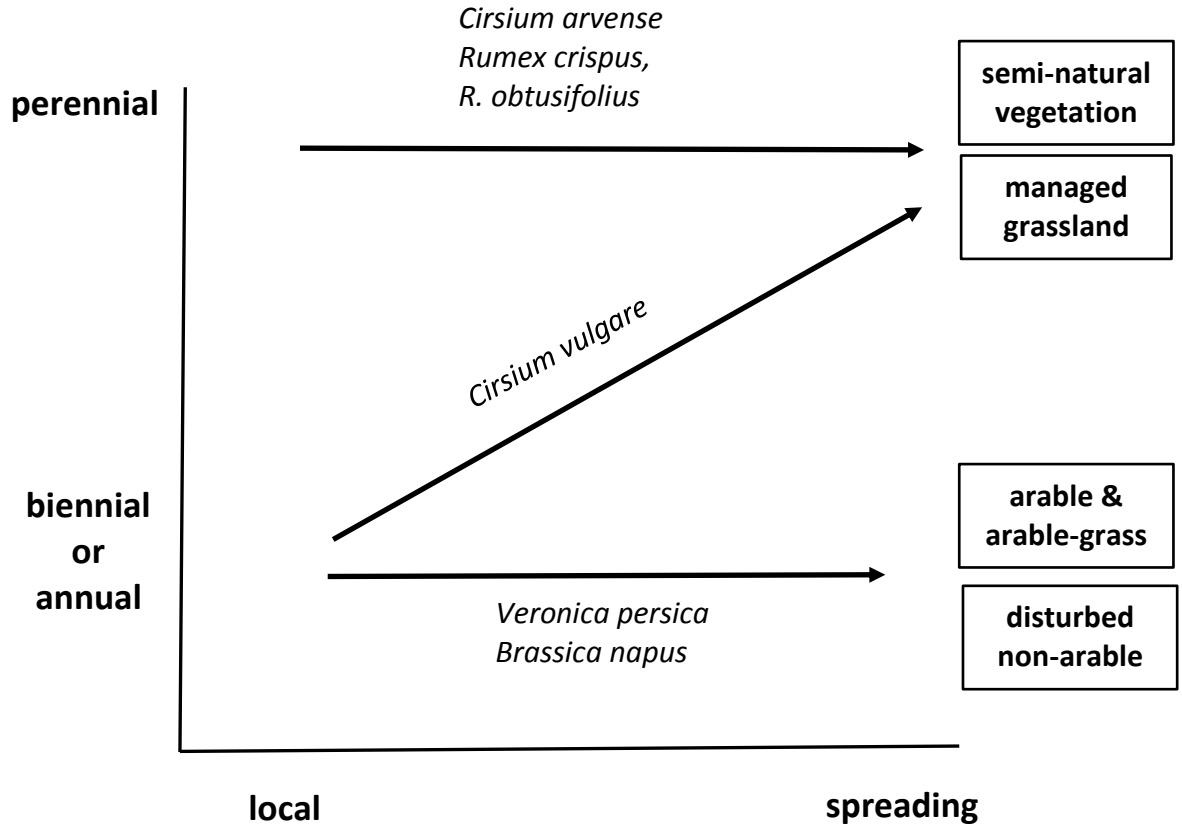


Figure 5.1 Diagram showing examples of plants in the UK having different growth habit that have spread from local or restricted habitats to occur over large tracts of land

To illustrate this, feral oilseed rape is compared with several of the major agricultural weeds in Figure 5.1. None of these plants were 'weeds' originally, but all have become serious weeds because they fit into the various cycles of grassland and arable land. All began at some time in local or restricted habitats (Salisbury, 1961).

The two docks, *Rumex crispus* and *R. obtusifolius*, and the creeping thistle, *Cirsium arvense*, are all native perennials that were restricted in the UK before agriculture provided an additional habitat for them. They became serious weeds of mixed stock and arable farming which dominated agriculture up to the 1960s (Brenchley, 1920; Long, 1938). These species and the biennial common thistle *Cirsium vulgare*, which took advantage of germination sites in arable and grazed farmland, became so troublesome in the first half of the 20th century that they were named in the *Weeds Acts* in that the landowners allowed them to flourish could be prosecuted. All four can grow in arable fields and disturbed, non-arable localities but are now readily controllable before flowering and so are no longer common in all-arable farms (see species lists in Debeljak *et al*. 2008; Hawes *et al.* 2010). Another perennial species that has spread throughout farmland is the North American *Epilobium adenocaulon* (previously *E. ciliatum*) first recorded in 1891 (Clapham *et al*. 1962).

The annual example, *Veronica persica*, was introduced from Asia and first recorded in the UK in 1825, but increased in the 20th century to become one of the commonest arable weeds through adapting its life cycle to the cropping practices and forming a viable seedbank (James Hutton Institute, seedbank archive). In relation to these species, oilseed rape is mainly an annual or winter annual. As a volunteer in arable fields, oilseed rape has become as common as *V. persica* in just a few decades (Debeljak *et al*. 2008; Clapham *et al*. 1962).

The potential of feral oilseed rape to become a major ruderal species, growing separate from but interacting with crops, is still uncertain however. Whether the

partial genetic isolation of the spring phenotypes from flowering crops will encourage local differentiation remains to be evaluated.

5.2.1 Comparison with other demographic studies in Europe

The EU project (SIGMEA) collated detailed information on feral oilseed rape and wild relatives in established agricultural habitats in several European countries. Comparison of a number of feral populations and crops per unit land area showed that regions surveyed in Germany, Denmark and the UK were similar in usually having less than one population per square kilometre and crops covering <10% of the arable surface (Squire *et al*. 2011). The exception was Selommes in central France, where both field areas and ferals occurred in greater area or number, respectively.

More detailed comparisons were made in SIGMEA of three established study areas in Selommes in France, Bremen in Germany and Tayside. This work does not form part of this thesis, but the 41 km^2 survey area in Selommes is shown in Figure 5.2 for comparison with representative areas in Tayside used in Chapter 4 (Figure 5.3). The ferals are seen to form more or less continuous strands along some stretches of road in Selommes, to the extent that spatially distinct populations were not usually visible. The stranding sometimes covered the entire length of one or two sides of a field, whereas in Tayside ferals do not generally occur in immediate proximity to current fields (Chapter 3). Ferals recurred at the same place more frequently at Selommes, sometimes present at the same place for up to 8 years, whereas populations seldom recurred at the same locations here (Chapter 3).

The reasons for differences between the study areas are not known. One possible factor is that the fields in Selommes had few clear boundaries between adjacent fields and roadsides, whereas those in Bremen and Tayside were separated with more physical boundaries such as hedges and walls. In Selommes, many ferals occurred immediately adjacent to fields, and could have arisen from pod shatter or local spillage in transport from the field directly to a road. In Tayside, very few fields occurred directly adjacent to a road without any boundary in between, so transport rarely occurred directly from a field to a road, but indirectly via farm tracks. The structure of fields, their boundaries and the roads serving agronomic operations might therefore play an important role in determining the location of ferals populations.

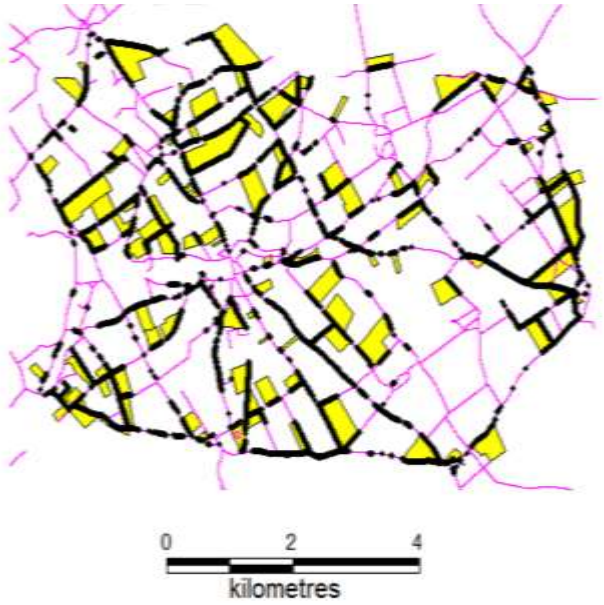


Figure 5.2 Agricultural region surveyed during the SIGMEA project. (France, Selommes 40 km^2), oilseed rape fields shown in yellow, feral oilseed rape shown by black stars. Taken from the SIGMEA report (Messéan et al. 2009) and associated databases

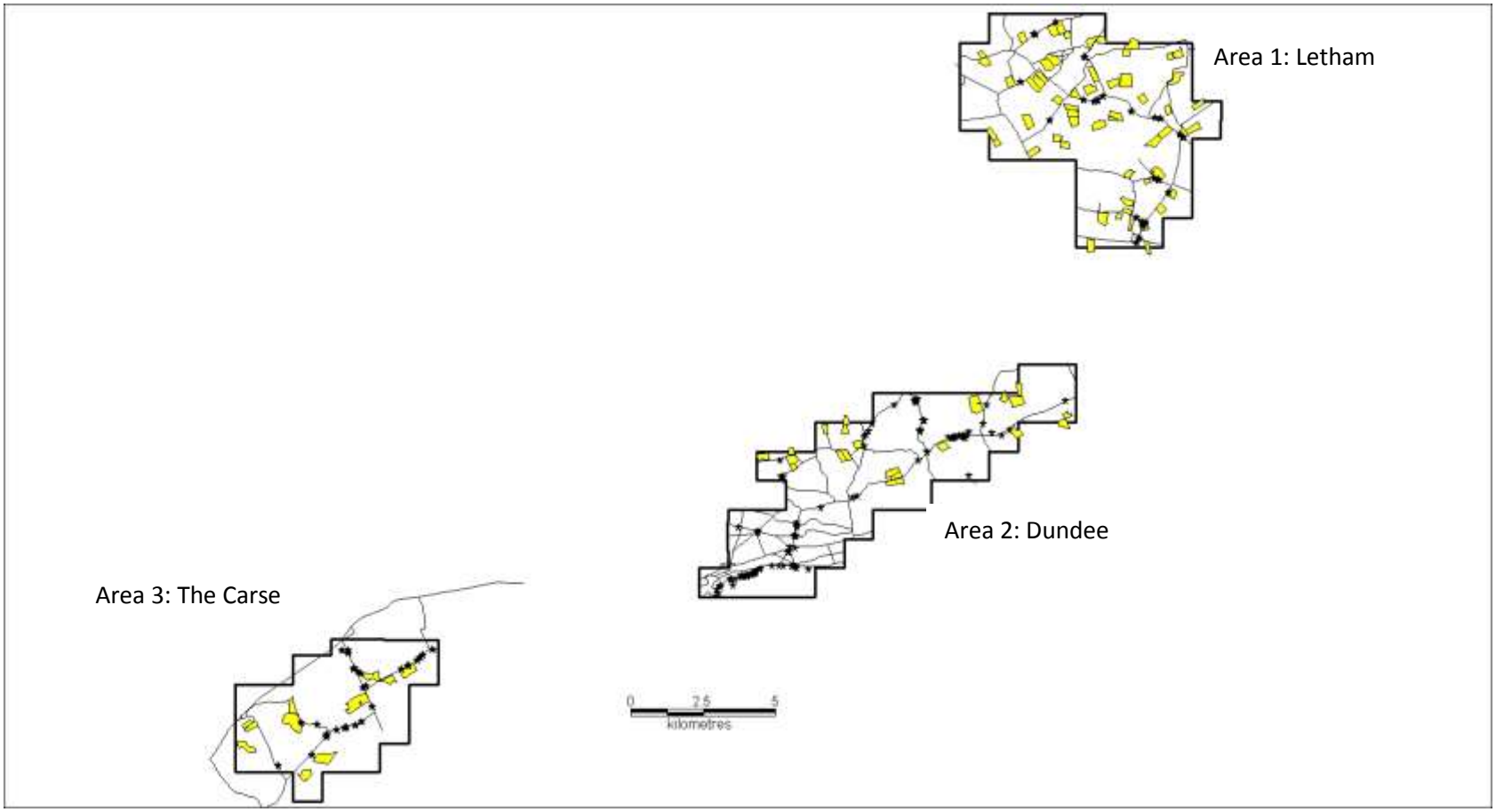


Figure 5.3 Three 40 km^2 agricultural regions in Tayside, oilseed rape fields shown in yellow, feral oilseed rape shown by black stars, roads as lines

Two other, more recent, studies of feral oilseed rape in Denmark (Mid-Jutland) and Germany (Braunschweig) also showed similar attributes to the Tayside survey. For example, these areas have a similar area of arable land as a percentage of the total land area and a similar frequency of feral sites (Squire *et al.* 2011). The Tayside area is therefore broadly representative of studies on feral oilseed rape within northern Europe. Selommes is the only study area to date in central Europe, so its representativeness is impossible to assess.

5.3 A platform and baseline for Environmental Risk Assessment (ERA)

The Tayside study has been considerably expanded in this thesis as a baseline and platform to aid the Environmental Risk Assessment of GM cropping. If GM crops were grown in the UK (or elsewhere) GM traits would become established in any resulting feral populations. The detailed knowledge of the Tayside study area would provide a realistic backdrop for considering whether any new traits, for example conferring resistance to abiotic stress (water shortage, low temperature) might lead to increase in population size of the ferals and to associated effects on wild populations and trophic webs.

5.3.1 Risk assessment procedures and guidelines

In Europe, the current approach to ERA for transgenic crops stems from the Directive 2001/18EC of the European Parliament and of the Council (2002) on the deliberate release into the environment of genetically modified organisms (GMOs). The environmental risk assessment must be performed according to the principles as laid down in the Directive. “The objective should” be, on a case by case basis, to identify and evaluate potential adverse effects, direct, or indirect, immediate or delayed, relating

to the release of a GMO in terms of human health and the environment (Henry *et al*. 2006). The ERA should be conducted with a view to identifying if there is a need for risk management to be associated with the release and if so, the most appropriate measures to be used. A general principle of the ERA is that analysis of the 'cumulative long-term effects' should also be carried out (Henry *et al.* 2006; BEETLE Report: ENV.B.3/ETU/2007/0007).

The EFSA 2010 guidance document is more comprehensive than formal risk assessments used by other countries. It asks that applicants (companies who wish to import or cultivate a GM crop) consider the steps in risk assessment (see Chapter One) for the following:

- Persistence and invasiveness
- Plant to micro-organism gene transfer
- Interaction of the GM plant with target organisms
- Interaction of the GM plant with target non-organisms
- Impact of specific cultivation associated with the GMO
- Effects on biogeochemical processes
- Effects on human and animal health

Depending on the intended uses of a GM plant, such as import for processing for food and feed and/or cultivation, the pathways and levels of exposure of the GM plant will vary (EFSA ERA 2010). If the use of the GM plant does not include cultivation in the EU, the problem formulation considers exposure via the accidental release into the environment (for example by seeds) during transportation and processing that could potentially lead to sporadic GM plants (i.e. ferals), and indirect exposure, for example

through manure and faeces from the gastrointestinal tracts mainly of animals fed the GM plant. It also considers organic matter which can be imported as a fertiliser or soil amendment or derived from other bioproducts of industrial processes. The stringency of the ERA can change when dealing with different situations. For example, it becomes more comprehensive when considering the release for cultivation than for assessing the risks of import. Even if a GMO has been assessed and is considered to be safe, EFSA recommends that further assessments may be necessary after commercialisation. The overall conclusions of the ERA should provide the basis for Post Market Environmental Monitoring (PMEM) that focuses on uncertainties in the potential risks identified in the ERA.

Once a risk assessment has been concluded and 'passed' by EFSA committees, member states may still put forward safeguard clauses to provisionally prohibit the placing on the market of authorised GMOs. For example, in 2009, Austria invoked Article 23 of Directive 2001/18/EC (safeguard) clause to provisionally prohibit the marketing of genetically modified rape on its territory. In several safeguard clauses, new data on the presence of ferals, either from import or cultivation, was the basis of the action. However, the new data was generally based on measurements over short time-scales of 1 to 3 years and for areas of limited geographical extent.

Even though the EFSA (2010) ERA guidelines emphasize that the potential for long term effects should be considered, a major limitation to current ERA is a shortage of long term data on impacts following the release of GM crops. This makes the Tayside Study a particularly valuable resource as a baseline and platform for testing current and developing new approaches to ERA. In the above list, ferals would be considered

relevant to three items: persistence and invasiveness, non-target effects and biogeochemical cycles.

5.3.2 Persistence and invasiveness

The procedure requires working through a series of questions and as indicated earlier (Chapter 2), the information which applicants can use to answer most of these questions is based on non-GM baseline data in Europe. The procedure consists of determining first the performance of conventional oilseed rape and then a judgement on whether the GM trait under consideration will cause plants to behave differently.

The first three questions as to whether the GM plant can grow, overwinter and reproduce and hybridise with wild relatives; all can be answered by known experiments and observations on conventional types. The next two examine the persistence and the fitness of the plant under agricultural conditions as a volunteer weed. The knowledge of oilseed rape GM and non-GM volunteers should provide sufficient background against which to assess a new trait (Messean *et al*. 2009). Knowledge of ferals becomes relevant to subsequent questions about whether the plant forms feral populations in the EU, and can hybridise with wild relatives outside agriculture. From the existing extensive research on ferals and wild relatives, both questions can be answered affirmatively. The original 3-year study in Tayside (Chapters 2, 3) provided some of the first data which was used to answer such questions.

The final questions have, however, come up against limited understanding. They ask whether a GM trait would affect the fitness, alter the range and cause change in population size of ferals or wild relatives, and then (outside the lower box) whether such

changes would lead to environmental harm, and could such harm be managed. Here, harm means, for example, loss of wild populations or habitat, interference with food webs or damage to iconic species. This study makes major contributions to arguments around these questions, yet in some respects, makes the analysis more difficult and problematic. The causes of the increase in feral populations here were not firmly identified. They may have included changing varietal characteristics, an evolution of greater fitness, a change in environment or a combination of these. However, the fact that spread and increase occurred would make it difficult (a) to predict whether a specific GM trait would itself lead to further spread or increase, and (b) to assess whether any spread or increase that occurred post-release of a GM crop was due to the particular GM trait or to unidentified factors that were independent of the genetic modification.

The study has, however, provided new information on whether GM ferals would cause damage to wild populations. The commonest ruderal Crucifer, charlock, had similar growth characteristics to feral oilseed rape, which suggests no marked advantage should come to the feral in terms of its intrinsic fitness (see 5.3.2). That ferals rarely occurred in exactly the same place charlock should result in very little inter-species competition. Within the Tayside study area there were many apparently suitable areas that were unoccupied by feral oilseed rape and charlock. The presence of feral oilseed rape actually brought about some potential benefits to food webs – more species, less dominance. While charlock and other wild crucifers are undergoing decline, they are doing so over much longer time scales than that over which feral oilseed rape has become prevalent (Preston *et al*. 2002). Finally, any attempts to control ferals, except on

major trunk roads, would be likely to cause more environmental damage than the presence of the ferals themselves (Devos *et al*. 2012).

5.3.3 Non-target effects (focal species)

Most non-target effects of GM crops have been examined in relation to GM crop plants growing in fields or depositing material such as pollen in large quantities on nearby plants that are fed on by non-target organisms (Poppy, 2000). Feralism, however, extends potential non-target effects outside fields. If the GM trait conferred herbicide tolerance, then the GM feral would have little effect unless the specific herbicide was used to control wayside vegetation. In Tayside, herbicides of any kind are not used for this purpose outside the main trunk roads.

If the trait was insect resistance, then GM ferals could be given an advantage through reduced herbivory. Any effect or advantage that the Bt ferals might have over non-Bt ferals would depend upon the particular Bt transformation involved, as different transformations confer resistance to different organisms. For example, Bt genes have been introduced into several brassica species (including oilseed rape), conferring resistance to the diamond back moth (*Plutella xylostella* (L.) (Shelton 2012). This moth originated in the Mediterranean region but has spread world-wide and causes large losses of yields. In the UK it has 2 generations per year, but in Australia it can have as many as 6-9 generations per year, leading to high pest pressure and severe losses in brassica crops. It can be hypothesised that if a pest such as this became more important in the UK, then the insect-resistant crop could be grown, and Bt feral oilseed rape would arise, possibly affecting non-target organisms such natural enemies. If Charlock

continues to decline further, non-target organisms might become more reliant on feral oilseed rape and so become more exposed to an insecticidal toxin.

Given there is usually a high number of non-target organisms that may be exposed to GM plants, current risk assessment methodology encourages researchers to define a representative sub-set of NTO species (referred to as 'focal species') for consideration in the risk assessment of each GM plant. A method proposed for identifying focal species (Andrade *et al.* 2012; EFSA-NTO 2010) is designed primarily for field crops but is here applied to ferals for the first time. Each non-target species is assessed as a potential focal species in relation to a range of ecological criteria. Scientific opinion (EFSA-NTO 2010) requires that at least one focal species is tested per relevant functional group, and that focal species are chosen for the various environments in which the GM crop will be grown.

The method of selecting focal species is now applied to invertebrates found on the Charlock and oilseed rape plants in Chapter 4 based on the scenario indicated above where GM resistance to Lepidopteran pests has been achieved. The following ecological criteria are used to rank the non-target species in EFSA-NTO 2010:

A – Species exposure to the GM plant under field conditions, specifically considering life stages present during the period exposure; for example, to what extent will the non-target species come into direct contact with GM plant material.

B – Known sensitivity of the species to the product(s) expressed in the GM plant; information usually collated from laboratory and contained studies.

C – Linkage to the production system (e.g. agro-ecosystem), and the presence of alternative food source; for example, is the non-target dependent on crops and does it have food sources elsewhere.

D – Abundance: is it likely to be found at sufficient abundance in most locations.

E – Species vulnerability: e.g. are certain populations already threatened and thus more vulnerable to additional pressures?

F – Relevance to adjacent habitats, including natural and semi-natural habitats: is any effect on the non-target likely to 'spill over' into non-agricultural habitats.

Scores are then calculated where 5 is the maximum effect and l is very little effect on the non-target organism. The average of the total score is taken and used as an indicator as to whether the organism is likely or not to be affected by the introduced GM trait. As examples, the exercise is shown for herbivores (Table 5.2) and natural enemies (Table 5.3).

Table 5.2 Criteria to be considered for herbivores as focal species (from EFSA-NTO 2010) applied to the invertebrate populations on feral oilseed rape

Ecological criteria	Flea Beetle	Snail	Auchenorrhyncha	Other weevils	Thrips	Sawfly	Aphids	Brassica pod midge	Cabbage Stem Beetle	Pollen Beetle	Lepidopteran Larvae	Cabbage seed weevil
A) Species exposure	5	5	5	5	5	5	5	5	5	5	5	5
B) Sensitivity	1	1	1	1	1	1	1	1	1	1	5	1
C) Linkage to production system	4	1	1	1	2	1	4	1	1	3	5	5
D) Abundance	1	1	1	1	4	1	4	4	1	5	3	2
E) Interactions with target species	1	1	1	1	1	1	3	1	1	1	5	1
F) Species vulnerability	1	1	1	1	1	3	1	1	1	1	1	1
G) Relevance to adjacent habitats	1	1	1	1	1	1	1	1	1	1	1	1
Average score	2.00	1.57	1.57	1.57	2.14	1.86	2.71	2.00	1.57	2.43	3.57	2.29

Table 5.3 Criteria to be considered for natural enemies as focal species applied to the invertebrate populations on feral oilseed rape

Ecological criteria	Ants	Beetles	Spiders	Rove Beetle	Parasitic wasp
A) Species exposure	3	3	3	3	3
B) Sensitivity	1	1	1	1	1
C) Linkage to production system	1	1	1	1	1
D) Abundance	1	1	1	1	1
E) Interactions with target species	1	3	1	1	1
F) Species vulnerability	1	1	1	1	1
G) Relevance to adjacent habitats	1	1	1	1	1
Average score	1.29	1.57	1.29	1.29	1.29

The exercise is inevitably subjective, but is transparent, can be readily amended through further discussion or as new data arise, and is increasingly used by stakeholder groups (S. Arpaie, personal communication). In this instance, the scores are low for both functional groups. For example, only one taxa is thought to be vulnerable – farmland sawflies, which have been reduced by high-intensity farm management. The groups are not closely tied to the production system in question because they can feed in other habitats and they do not strongly impinge on other habitats around agriculture. Some of the insects were found at low abundance and therefore are not ideal candidates for focal species.

In Table 5.2, the herbivores that the GM trait might be most likely to affect would be non-target Lepidopteran larvae and aphids. Some of the larvae are already pests, so the analysis points to the need for further toxicological work to assess the relative susceptibility of target and non-target larvae. For example, reducing the target pest might allow non-targets to increase. For natural enemies, the most likely insects to be affected by the GM trait would be carabid beetles which prey on the non-target Lepidopteran larvae and also the target species.

The approach to focal species taken in Tables 5.2 and 5.3 is indicative of what can be achieved with knowledge of the invertebrates growing on feral oilseed rape and related plants. It could be modified to take account of a particular GM trait that was about to be introduced.

5.3.4 Biogeochemical cycles and other assessment categories

The cycling of elements such as carbon, nitrogen and phosphorus are considered as a risk-category following the 2010 revision of the EFSA ERA Guidelines. Novel crop plants could pose a risk if, for example, they required different amounts of fertiliser or altered processes such as nitrogen fixation, denitrification or decomposition. Feral plants must interact with the cycles of energy and elements because (like all plants) since they need light and nutrients for their survival and growth. Simply affecting a process, however, does not constitute a risk: the effect has to be assessed in relation to other factors that normally impinge on the process.

Any effects of ferals have to be viewed in relation to the background dynamics in the processes. Much data (not presented here) is available for the inputs of nitrogen and other mineral elements into the Tayside agro-ecosystems as fertiliser and atmospheric deposition and for offtake in yield of crops and animals. Given, however, their low degree of invasiveness and low population density, any effect of ferals on biogeochemical cycles would be several orders of magnitude smaller than that of crop plants or volunteers. An impact on biogeochemical cycles due to GM ferals, comparable to the impacts associated with agricultural production itself, is therefore likely to be negligible at their present population density.

Ferals would also be of limited relevance for other risk categories. The transfer of plant genes to microorganisms would have been considered already with respect to the GM plant. Ecological effects such as spread of secondary pests resulting from reduction in target organisms could be informed by some of the findings here on the harbouring of pests by ferals. The only potential impact through change in management after a GM

crop was introduced would be if ferals themselves were managed, for example by spraying herbicide on roadside vegetation, as part of measures to achieve GM coexistence. The consequent effects on increased run-off of rainwater or soil erosion would be far greater than any effects of the ferals themselves. There is, however, no scientific justification for managing ferals to achieve coexistence (Devos *et al*. 2012; Chapter 2). Finally, ferals contribute a negligible amount to harvested crops so would not be relevant to risk to human or animal health.

5.4 Conclusions and further work

Value of long term comprehensive studies

This long term study has altered the initial understanding of the consequences of feral populations in oilseed rape. During the three years from 1993-1995 the Tayside survey did not (and could not) predict the subsequent increase in ferals. The eleven year survey provides a case for using long term monitoring, particularly when there is a change in context (in the Tayside study a decline in crop and an increase in feral populations). It is likely therefore that there will be unpredicted consequences that would emerge from post-release interactions for which short term risk assessments are unlikely to provide the answers.

Although science needs to investigate the long term impacts of any new organisms that are released into the environment, it must arguably also take a lead in the interpretation of the results and implications. Data showing the persistence of feral oilseed rape has led to some countries invoking a safeguard clause to prevent cultivation of certain crops on their territory. For example, Greece invoked a safe guard clause to prevent

genetically modified maize being placed on the market (MON 810 according to Article 23 of Directive 2001/18/EC EFSA Journal 2012 (www.efsa.europa.eu/efsajournal).
Under the European GMO legal framework, Member States may invoke the safeguard clause if justified by relevant scientific evidence such as a new report on the persistence of ferals. EFSA can be called on by the European Commission to assess this scientific evidence in relation to a GMO it has already evaluated. This can lead to delays which can take months to resolve.

Although this long term study has shown ferals to increase and persist, as they have had very little impact in the ecological scene, there is no reason to suppose they constitute a risk, and therefore no reason to justify any action at the present time. The fact that ferals are widely distributed, and highly variable in location from year to year, would make it very difficult in any case to formulate and apply control measures.

Several studies of feral oilseed rape have used molecular markers and techniques to distinguish between different populations (DETR, 1999; Cuthbert and McVetty 2001; Pascher *et. al* 2010). Such markers have sometimes been helpful in assessing the origin and spread of ferals. Molecular analysis was not used in this thesis because many different varieties of oilseed rape had been grown within Tayside for many years, making it difficult to assess where exactly the feral oilseed rape had originated from. If the spread of new varieties of oilseed rape was to be considered by using molecular methods, then the cultivar used would need to be readily distinguished from cultivars already in existence, and the feral populations would need to be followed over a long period of time with plant samples needing to be taken over an extensive area. This

would involve a considerable amount of time and effort and would not necessarily allow definition of origin.

Feral oilseed rape populations in 2012

A rapid survey was undertaken in 2012, mapping oilseed rape crops and feral plants within 400 km^2 of Tayside. There were 222 winter sown oilseed rape fields and only 2 spring sown fields. Interestingly, due to low temperature, the oilseed rape fields were in full flower for around 6 weeks which was approximately 2 weeks longer than would normally be expected. There were very few (7) feral populations flowering at the same time as the fields, but around 80 volunteer populations were visible. The majority of the feral oilseed rape appeared in larger numbers after the crop had flowered, reinforcing the conclusion that feral oilseed rape is becoming differentiated in its developmental timing from oilseed rape crops.

Main Conclusions

- This is the first study globally to demonstrate persistence, spread and increase of feral oilseed rape in a large agricultural area for a duration of over 10 years. While individual populations rapidly go extinct, the population as a whole increased at the landscape scale through the continued occupation of new sites.
- The spread of ferals into farmland, and notably the continued presence of spring varieties with little pollen contact with fields, point to ferals as being able to maintain phenotypes which are dissimilar to most current crops.
- The causes of spread into new farmland areas (e.g. by redistribution of seed or new spilage) were not distinguished in this study. Molecular techniques and phenotypic

screening for traits such as flowering without the need for vernalisation could elucidate some of the remaining questions on the means of spread.

- Feral oilseed rape has an ecological role that would vary with locality (region or country) In Tayside, its ecological role was judged to be small: the insect species present on it are the same species that inhabit local similar crucifers.
- Although there are currently no existing implications for crop coexistence because there are not enough ferals in the Tayside region compared to crop plants, the numbers should still be periodically be monitored as this survey has shown that feral oilseed rape can increase even though the cropped area has declined.
- This study has clearly shown that the results from short term surveys are unlikely to be a useful predictor of population spread, particularly in situations where the context changes.
- The study offers the potential to be used as a baseline data set for modelling the impacts of GM crops through ferals and their contact with wild crucifers.

References

Alexander, H.M., Pilson D., Moody-Weis J., & Slade N.A. (2009) Geographic variation in dynamics of an annual plant with a seed bank. *Journal of Ecology* 97: 1390-1400

Alford, D. V. (2003) Biocontrol of Oilseed Rape Pests. Blackwell Publishing. Oxford. U.K 1-355

Alford, L., Blackburn, T.M., & Bale, J.S. (2011) Effects of acclimation and latitude on the activity thresholds of the aphid Myzus persicae in Europe. *Journal of Applied Entomology* 135: 332-346

Allendorf, F.W. & Lundquist, L.L. (2003) Introduction: Population Biology, Evolution, and Control of Invasive Species. *Conservation Biology* 17: 24-30

Andersen, N.S., Rasmussen, J., & Jørgensen, R.B. (2010) You reap what you sow – or do you? – volunteers in organic row-sown and broadcast-sown oilseed rape fields. *European Journal of Agronomy* 32: 121-126

Andrade, C., Chiron, F., & Julliard, R. (2012) Improving the selection of focal species exposed to pesticides to support ecological risk assessments. *Ecotoxicology* 21: 2430-2440

Bagavathiannan, M.V. & Van Acker, R.C. (2008) Crop ferality: Implications for novel trait confinement. *Agriculture, Ecosystems & Environment* 127: 1-6

Bagavathiannan, M.V., Begg, G.S., Gulden, R.H., & Van Acker, R.C. (2012) Modelling the Dynamics of Feral Alfalfa Populations and Its Management Implications. *PLoS ONE* 7: e39440-

Bartsch, D., Buhk, H. J., Gathmann, A., Koziolek, C., Meisner, A., Neemann, G., Rees, U., Schulte, E., & Vaasen, A. (2007) *BEETLE Report: Long term effects of genetically modified (GM) crops on health, biodiversity and the environment: prioritisation of potential risks and delimitation of uncertainties*. 1-132

Beckie, H.J. & Warwick, S.I. (2010) Persistence of an oilseed rape transgene in the environment. *Crop Protection* 29: 509-512

Begg, G.S., Hockaday, S., McNicol, J.W., Askew, M., & Squire, G.R. (2006) Modelling the persistence of volunteer oilseed rape (*Brassica napus*). *Ecological Modelling* 198: 195-207

Begon, M., Harper, J. L., & Townsend, C. R. (1996) "Ecology: Individuals, populations, and communities," Third ed. Blackwell Science Ltd, Oxford. U.K. pp. 334-335.

Birch, A., Griffiths, B., Caul, S., Thompson, J., Heckmann, L., Krogh, P., & Cortet, J. (2007) The role of laboratory, glasshouse and field scale experiments in understanding the interactions between genetically modified crops and soil ecosystems: a review of the ECOGEN project. *Pedobiologia* 51: 251-260

Brenchley, W. E. (1920) Weeds of farmland. Longmans. London. 1-270

Brown, G.S. (1965) Point density in stems per acre. *New Zealand Forestry Service Research Notes* 38: 1-11

Bruce, T.J.A. (2006) Tackling the threat to food security caused by crop pests in the new millennium. *Food Security* 2: 133-141

Bukovinszky, T., van Veen, F.J.F., Jongema, Y., & Dicke, M. (2008) Direct and Indirect Effects of Resource Quality on Food Web Structure
104. *Science* 319: 804-807

Chapman, M.A. & Burke, J.M. (2006) Letting the gene out of the bottle: the population genetics of genetically modified crops. *New Phytologist* 170: 429-443

Charters, Y. M., Robertson, A., & Squire, G. R. (1999) *Investigation of feral oilseed rape populations. Genetically modified organisms research report (No. 12)*, Department of the Environment, Transport and Regions, London. 1-93

Charters, Y.M., Robertson, A., Wilkinson, M.J., & Ramsay, G. (1996) PCR analysis of oilseed rape cultivars (*Brassica napus* L ssp oleifera) using 5'-anchored simple sequence repeat (SSR) primers. *Theoretical and Applied Genetics* 92: 442-447

Chew, F.S. & Courtney, S.P. (1991) Plant Apparency and Evolutionary Escape from Insect Herbivory. *The American Naturalist* 138: 729-750

Chèvre, A.M., Eber, F., Baranger, A., Hureau, G., Barret, P., Picault, H., & Renard, M. (1998) Characterization of backcross generations obtained under field conditions from oilseed rape-wild radish F1 interspecific hybrids: an assessment of transgene dispersal. *TAG Theoretical and Applied Genetics* 97: 90-98

Claessen, D., Gilligan, C.A., & Bosch, F. (2005) Which traits promote persistence of feral GM crops? Part 2: implications of metapopulation structure. *Oikos* 110: 30-42

Clapham, A. R., Tutin, T. G., & Warburg, E. F. (1962) "Flora of the British Isles," Cambridge University Press.

Colwell, R.K., Mao, C.X., & Chang, J. (2004) Interpolating,extrapolating, and comparing incidence-based species accumulation curves. *Ecology* 85: 2717-2727

Crawley, M.J., Hails, R.S., Rees, M., Kohn, D., & Buxton, J. (1993) Ecology of transgenic oilseed rape in natural habitats. *Nature* 363: 620-623

Crawley, M.J. & Brown, S.L. (1995) Seed Limitation and the Dynamics of Feral Oilseed Rape on the M25 Motorway. *Proceedings of the Royal Society of London.Series B: Biological Sciences* 259: 49-54

Crawley, M.J. & Brown, S.L. (2004) Spatially structured population dynamics in feral oilseed rape. *Proceedings of the Royal Society of London Series B-Biological Sciences* 271: 1909-1916

Cullen, D.W., Squire, G.R., McNicol, J.W., Jacobs, J.H., Osborne, J.L., Ford, L., Ramsay, G., Scrimgeour, C., & Young, M.W. (2008) Development and validation of gas chromatography and real-time quantitative PCR for the quantification of landscape-scale gene flow from varieties of high erucic acid (HEAR) oilseed rape. *Journal of the Science of Food and Agriculture* 88: 2253-2264

Cuthbert, J.L. & McVetty, E. (2001) Plot-to-plot, row-to-row and plant-to-plant outcrossing studies in oilseed rape. *Canadian Journal of Plant Science* 81: 657-664

Damgaard, C. & Kjellsson, G. (2005) Gene flow of oilseed rape (*Brassica napus*) according to isolation distance and buffer zone. *Agriculture, Ecosystems & Environment* 108: 291-301

Debeljak, M., Squire, G.R., Demsar, D., Young, M.W., & Dzeroski, S. (2008) Relations between the oilseed rape volunteer seedbank, and soil factors, weed functional groups and geographical location in the UK. *Ecological Modelling* 212: 138-146

Defra (2010). Fertiliser Manual (RB209) 8th Edition. www.defra.gov.uk/publications/files/rb209-fertiliser-manual-110412.pdf 115-117

Defra (2010). Pesticide usage survey Report 235. Arable crops in the United Kingdom. www.fera.defra.gov.uk/scienceResearch/science/lus/documents/arable2010.pdf. 29-32

Defra (2011). UK cereal and oilseed areas, yields and production - datasheet. http://www.defra.gov.uk/statistics/foodfarm/food/cereals/cerealsoilseed 1-1

Devaux, C., Lavigne, C., Falentin-Guyomarc'h, H., Vautrin, S., Lecomte, J., & Klein, E.K. (2005) High diversity of oilseed rape pollen clouds over an agro-ecosystem indicates long-distance dispersal. *Molecular Ecology* 14: 2269-2280

Devaux, C., Klein, E.K., Lavigne, C., Sausse, C., & Messean, A. (2008) Environmental and landscape effects on cross-pollination rates observed at long distance among French oilseed rape *Brassica napus* commercial fields. *Journal of Applied Ecology* 45: 803-812

Devos, Y., Reheul, D., Schrijver, A.D.E., Cors, F., & Moens, W. (2004) Management of herbicide-tolerant oilseed rape in Europe: a case study on minimizing vertical gene flow. *Environmental Biosafety Research* 3: 135-148

Devos, Y., Hails, R.S., Messéan, A., Perry, J.N., & Squire, G.R. (2012) Feral genetically modified herbicide tolerant oilseed rape from seed import spills: are concerns scientifically justified? *Transgenic Research* 21: 1-21

Di, K., Stewart, C.N., Wei, W., Shen, B.C., Tang, Z.X., & Ma, K.P. (2009) Fitness and maternal effects in hybrids formed between transgenic oilseed rape (*Brassica napus* L.) and wild brown mustard [*B. juncea* (L.) Czern et Coss.] in the field. *Pest Management Science* 65: 753-760

Diaz, S., Hector, A., & Wardle, D.A. (2009) Biodiversity in forest carbon sequestration initiatives: not just a side benefit. *Current Opinion in Environmental Sustainability* 1: 55-60

Didham, R.K., Tylianakis, J.M., Gemmell, N.J., Rand, T.A., & Ewers, R.M. (2007) Interactive effects of habitat modification and species invasion on native species decline. *Trends in Ecology & Evolution* 22: 489-496

Dietz-Pfeilstetter, A., Metge, K., Schönfeld, J., & Zwerger, P. (2006) Assessment of transgene spread from oilseed rape by population dynamic and molecular analyses of feral oilseed rape. *Journal of Plant Diseases and Protection 20 [Special issue]*: 39-47

EC (2003) Regulation (EC) 1829/2003 of the European Parliament and the Council of 22 September 2003 on genetically modified food and feed. *Journal European Commisson* L268: 1-23

EFSA (2010) EFSA Panel on Genetically Modified Organisms (GMO); Guidance on the environmental risk assessment of genetically modified plants. *EFSA Journal 2010 8(11):1879*: 1-111

Ekbom, B. & Müller, A. (2011) Flea beetle (*Phyllotreta undulata* Kutschera) sensitivity to insecticides used in seed dressings and foliar sprays. *Crop Protection* 30: 1376-1379

Elling, B., Neuffer, B., & Bleeker, W. (2009) Sources of genetic diversity in feral oilseed rape (*Brassica napus*) populations. *Basic and Applied Ecology* 10: 544-553

Elling, B., Hochkirch, M., Neuffer, B., & Bleeker, W. (2010) Hybridisation between oilseed rape (*Brassica napus*) and tetraploid *Brassica rapa* under field conditions. *Flora - Morphology, Distribution, Functional Ecology of Plants* 205: 411-417

Ellstrand, N.C., Prentice, H.C., & Hancock, J.F. (1999) Gene flow and introgression from domesticated plants into their wild relatives. *Annual Review of Ecology and Systematics* 30: 539-563

Firbank, L.G., Heard, M.S., Woiwod, I.P., Hawes, C., Haughton, A.J., Champion, G.T., Scott, R.J., Hill, M.O., Dewar, A.M., Squire, G.R., May, M.J., Brooks, D.R., Bohan, D.A., Daniels, R.E., Osborne, J.L., Roy, D.B., Black, H.I.J., Rothery, P., & Perry, J.N. (2003) An introduction to the Farm-Scale Evaluations of genetically modified herbicide-tolerant crops. *Journal of Applied Ecology* 40: 2-16

FitzJohn, R.G., Armstrong, T.T., Newstrom-Lloyd, L.E., Wilton, A.D., & Cochrane, M. Hybridisation within Brassica and allied genera: evaluation of potential for transgene escape. *Euphytica* 158: 209-230

Ford, C.S., Allainguillaume, J., Grilli-Chantler, P., Cuccato, G., Allender, C.J., & Wilkinson, M.J. (2006) Spontaneous gene flow from rapeseed (*Brassica napus*) to wild *Brassica oleracea*. *Proceedings of the Royal Society B-Biological Sciences* 273: 3111-3115

Freckleton, R.P. & Watkinson, A.R. (2002) Large-scale spatial dynamics of plants: metapopulations, regional ensembles and patchy populations. *Journal of Ecology* 90: 419-434

Garnier, A. & Lecomte, J. (2006) Using a spatial and stage-structured invasion model to assess the spread of feral populations of transgenic oilseed rape 10. *Ecological Modelling* 194: 141-149

Garnier, A., Pivard, S., & Lecomte, J. (2008) Measuring and modelling anthropogenic secondary seed dispersal along roadverges for feral oilseed rape. *Basic and Applied Ecology* 9: 533-541

Gols, R., Bukovinszky, T., van Dam, N.M., Dicke, M., Bullock, J.M., & Harvey, J.A. (2008) Performance of generalist and specialist herbivores and their endoparasitoids differs on cultivated and wild Brassica populations. *Journal of Chemical Ecology* 34: 132-143

Gressel, J. (2005) "Introduction - The Challenges of Ferality," *In Crop ferality and volunteerism*, J. Gressel, ed., CRC Press, Florida. pp. 26-42.

Gruber, S., Pekrun, C., & Claupein, W. (2004) Population dynamics of volunteer oilseed rape (*Brassica napus L.*) affected by tillage. *European Journal of Agronomy* 20: 351-361

Gruber, S., Pekrun, C., & Claupein, W. (2005) Life cycle and potential gene flow of volunteer oilseed rape in different tillage systems. *Weed Research* 45: 83-93

Gruber, S. & Claupein, W. (2008) Emergence and establishment of volunteer oilseed rape in spring and winter crops. *Journal of Plant Diseases and Protection*: 193-198

Gruber, S., Bühler, A., Möhring, J., & Claupein, W. (2010) Sleepers in the soil – Vertical distribution by tillage and long-term survival of oilseed rape seeds

compared with plastic pellets
106. *European Journal of Agronomy* 33: 81-88

Gueritaine, G. & Darmency, H. (2001) Polymorphism for interspecific hybridisation within a population of wild radish (*Raphanus raphanistrum*) pollinated by oilseed rape (*Brassica napus*). *Sexual Plant Reproduction* 14: 169-172

Gueritaine, G., Bazot, S., & Darmency, H. (2003) Emergence and growth of hybrids between *Brassica napus* and *Raphanus raphanistrum*. *The New phytologist* 158: 561-567

Guggisberg, A., Welk, E., Sforza, R., Horvath, D.P., Anderson, J.V., Foley, M.E., & Rieseberg, L.H. (2012) Invasion history of North American Canada thistle, *Cirsium arvense*. *Journal of Biogeography* 39: 1919-1931

Gutbrodt, B., Dorn, S., Unsicker, S.B., & Mody, K. (2012) Species-specific responses of herbivores to within-plant and environmentally mediated between-plant variability in plant chemistry. *Chemoecology* 22: 101-111

Hansen, L.B., Siegismund, H.R., & Jørgensen, R.B. (2003) Progressive introgression between *Brassica napus* (oilseed rape) and *B. rapa*. *Heredity* 91: 276-283

Harrison, S. K. & Taylor, A. D. (1997) "Empirical evidence for metapopulation dynamics. Metapopulation Biology: Ecology, Genetics and Evolution," Academic Press, London. pp. 27-42.

Hauser, T.P., Damgaard, C., & Jørgensen, R.B. (2003) Frequency-dependent fitness of hybrids between oilseed rape (*Brassica napus*) and weedy *B. rapa* (Brassicaceae). *American Journal of Botany* 90: 571-578

Hawes, C., Haughton, A.J., Osborne, J.L., Roy, D.B., Clark, S.J., Perry, J.N., Rothery, P., Bohan, D.A., Brooks, D.R., Champion, G.T., Dewar, A.M., Heard, M.S., Woiwod, I.P., Daniels, R.E., Young, M.W., Parish, A.M., Scott, R.J., Firbank, L.G., & Squire, G.R. (2003) Responses of plants and invertebrate trophic groups to contrasting herbicide regimes in the Farm Scale Evaluations of genetically modified herbicide-tolerant crops. *Philosophical Transactions of The Royal Society B* 358: 1899-1913

Hawes, C., Haughton, A.J., Bohan, D.A., & Squire, G.R. (2009) Functional approaches for assessing plant and invertebrate abundance patterns in arable systems. *Basic and Applied Ecology* 10: 34-42

Hawes, C., Squire, G.R., Hallett, P.D., Watson, C.A., & Young, M. (2010) Arable plant communities as indicators of farming practice. *Agriculture Ecosystems & Environment* 138: 17-26

Heard, M.S., Hawes, C., Champion, G.T., Clark, S.J., Firbank, L.G., Haughton, A.J., Parish, A.M., Perry, J.N., Rothery, P., Scott, R.J., Skellern, M.P., Squire, G.R., & Hill,

M.O. (2003) Weeds in fields with contrasting conventional and genetically modified herbicide-tolerant crops. 1. Effects on abundance and diversity. *Philosophical Transactions of The Royal Society B* 358: 1819-1832

Henry, C., Hugo, S., Blackburn, J., Central Science Laboratory (CSL), Nicholls, D., & RM Consultants Ltd. (2006) *Cumulative long-term effects of genetically modified (GM) crops on human/animal health an the environment: risk assessment methodologies Reference: No 07-0402/2005/414455/MAR/B4*. 1-236

Hopkins, R.J., van Dam, N.M., & van Loon, J.J.A. (2008) Role of Glucosinolates in Insect-Plant Relationships and Multitrophic Interactions. *Annual Review of Entomology* 54: 57-83

Hughes, J.B., Hellmann, J.J., Ricketts, T.H., & Bohannan, B.J.M. (2001) Counting the uncountable: Statistical approaches to estimating microbial diversity. *Applied and Environmental Microbiology* 67: 4399-4406

James, C. Global Status of Commercialized Biotech/GM Crops: (2011). ISAAA Brief 43. Ithaca, New York.

Johnson, K.L., Raybould, A.F., Hudson, M.D., & Poppy, G.M. (2007) How does scientific risk assessment of GM crops fit within the wider risk analysis? *Trends in Plant Science* 12: 1-5

Jørgensen, R.B. & Anderson, B. (1994) Spontaneous hybridization between oilseed rape (*Brassica napus*) and weedy *B. campestris* (Brassicaceae): A risk of growing genetically modified oilseed rape. *American Journal of Botany* 81: 1620-1626

Jørgensen, R.B. (2007) Oilseed Rape: Co-existence and Gene Flow from Wild Species. *Advances in Botanical Research: Incorporating Advances in Plant Pathology* 45: 451-464

Kawata, M., Murakami, K., & Ishikawa, T. (2009) Dispersal and persistence of genetically modified oilseed rape around Japanese harbors. *Environmental Science and Pollution Research* 16: 120-126

Kimber, D. (1981) "Varieties," *In Oilseed Rape - A manual for growers, farmers and advisors*, Cambridge Agricultural Publishing, Ipswich. pp. 41-51.

Knispel, A. & McLachlan, S. (2010) Landscape-scale distribution and persistence of genetically modified oilseed rape (*Brassica napus*) in Manitoba, Canada. *Environmental Science and Pollution Research* 17: 13-25

Knopf, A. (2001) "National Audubon Field Guide to North American Wildflowers, Eastern Region," 2nd Edition ed. New York.

Krymañski, J. (1998) Agronomy of Oilseed Brassicas. *International Symposium Brassica 97, Xth Crucifer Genetics Workshop*: 55-60

Landbo, L., Andersen, B., & Jørgensen, R.B. (1996) Natural Hybridisation between Oilseed Rape and a Wild Relative: Hybrids among Seeds from Weedy *B. Campestris*. *Hereditas* 125: 89-91

Lefol, E., SeguinSwarts, G., & Downey, R.K. (1997) Sexual hybridisation in crosses of cultivated Brassica species with the crucifers Erucastrum gallicum and Raphanus raphanistrum: Potential for gene introgression. *Euphytica* 95: 127-139

Leigh, R. A. and Johnston, A. E. (1994) Long term experiments in Agricultural and Ecological Sciences. University Press. Cambridge. 3-421

Long, H. C. (1938) *Weeds of arable land,* HMSO, London MAFF Bulletin 108. -

Lu, B.R. & Yang, C. (2009) Gene flow from genetically modified rice to its wild relatives: Assessing potential ecological consequences. *Biotechnology Advances* 27: 1083-1091

Luijten, S. H. & de Jong, T. (2010) *A baseline study of the distribution and morphology of Brassica napus L. and Brassica rapa L. in the Netherlands,* Leiden University, Netherlands CGM 2010-3. -

Lutman, P.J.W., Freeman, S.E., & Pekrun, C. (2003) The long-term persistence of seeds of oilseed rape (*Brassica napus*) in arable fields. *Journal of Agricultural Science* 141: 231-240

Magurran, A. E. (2004) "Measuring Biological Diversity," Blackwell Publishing, Oxford. pp. 19-130.

Main, A. R. (1982) Rare species: Precious or dross? In species at risk: Research in Australia, *In Proceedings of a Symposium on the Biology of Rare and Endangered Species in Australia*, R. H. Groves & W. D. L. Ride, eds..

Marshall, B. & Squire, G.R. (1996) Non-linearity in rate-temperature relations of germination in oilseed rape. *Journal of Experimental Botany* 47: 1369-1375

Marshall, B., Dunlop, G., Ramsay, G., & Squire, G.R. (2000) Temperature-dependent germination traits in oilseed rape associated with 5'- anchored simple sequence repeat PCR polymorphisms. *Journal of Experimental Botany* 51: 2075-2084

McGinley, M. & Emmett Duffy, J. (2010) Species Richness. *Encyclopedia of Earth - environmental Information Coalition, National Council for Science and Environment, Washington DC*: 1-132

MEAD, R. (1966) A Relationship between Individual Plant-spacing and Yield. *Annals of Botany* 30: 301-309

Menzel, G. (2006) *Verbreitungsdynamik und Auskreuzungspotential von Brassica napus L. (Raps) im Grossraum Bremen.,* GCA-Verlag, Waabs, Bremen. -

Messéan, A., Squire, G.R., Perry, J.N., Angevin, F., Gómez-Barbero, M., Townend, D., Sausse, C., Breckling, B., Langrell, S., Dzeroski, S., & Sweet, J.B. (2009) Sustainable Introduction of GM crops into european agriculture: a summary report of the FP6 SIGMEA research project. *Oléagineux, Corps Gras, Lipides* 16: 37-51

Messéan, A. SIGMEA Final Report. http://www.inra.fr.sigmea/final_report.

Middelhoff, U., Reuter, H., & Breckling, B. (2011) GeneTraMP, a spatio-temporal model of the dispersal and persistence of transgenes in feral, volunteer and crop plants of oilseed rape and related species. *Ecological Indicators* 11: 974-988

Moyes, C.L., Lilley, J.M., Casais, C.A., Cole, S.G., Haeger, P.D., & Dale, P.J. (2002) Barriers to gene flow from oilseed rape (*Brassica napus*) into populations of *Sinapis arvensis*. *Molecular Ecology* 11: 103-112

Orlovius, K. (2003) Fertilizing for High yield and Quality. *IPI Bulletin 16*: 1-130

Pascher, K., Macalka, S., Rau, D., Gollmann, G., Reiner, H., Glossl, J., & Grabherr, G. (2010) Molecular differentiation of commercial varieties and feral populations of oilseed rape (*Brassica napus L.*). *BMC Evolutionary Biology* 10: 63-

Pekrun, C., Lane, P.W., & Lutman, P.J.W. (2005) Modelling seedbank dynamics of volunteer oilseed rape (*Brassica napus*). *Agricultural Systems* 84: 1-20

Pekrun, C., Lutman, P.J.W., Büchse, A., Albertini, A., & Claupein, W. (2006) Reducing potential gene escape in time by appropriate post-harvest tillage − Evidence from field experiments with oilseed rape at 10 sites in Europe. *European Journal of Agronomy* 25: 289-298

Peltzer, D.A., Ferriss, S., & FitzJohn, R.G. (2008) Predicting weed distribution at the landscape scale: using naturalized Brassica as a model system. *Journal Applied Ecology* 45: 467-475

Pessel, F.D., Lecomte, J., Emeriau, V., Krouti, M., Messean, A., & Gouyon, P.H. (2001) Persistence of oilseed rape (*Brassica napus L.*) outside of cultivated fields. *TAG.Theoretical and applied genetics.Theoretische und angewandte Genetik* 102: 841-846

Pivard, S., Adamczyk, K., Lecomte, J., Lavigne, C., Bouvier, A., Deville, A., Gouyon, P.H., & Huet, S. (2008) Where do the feral oilseed rape populations come from? A large-scale study of their possible origin in a farmland area. *Journal of Applied Ecology* 45: 476-485

Poelman, E.H., Galiart, R.J.F.H., Raaijmakers, C.E., van Loon, J.J.A., & van Dam, N.M. (2008) Performance of specialist and generalist herbivores feeding on cabbage cultivars is not explained by glucosinolate profiles. *Entomologia Experimentalis et Applicata* 127: 218-228

Poppy, G. (2000) GM crops: environmental risks and non-target effects. *Trends in Plant Science* 5: 4-6

Poppy, G.M. (2004) Geneflow from GM plants – towards a more quantitative risk assessment. *Trends in Biotechnology* 22: 436-438

Preston, C. D., Pearman, D. A., and Dines, T. D (2002) New Atlas of the British and Irish Flora. Oxford University Press. Hong Kong 39-281

Raman, H., Raman, R., Nelson, M.N., Aslam, M.N., Rajasekaran, R., Wratten, N., Cowling, W.A., Kilian, A., Sharpe, A.G., & Schondelmaier, J. (2012) Diversity Array Technology Markers: Genetic Diversity Analyses and Linkage Map Construction in Rapeseed (*Brassica napus* L.). *DNA Research* 19: 51-65

Ramsay, G., Thompson, C., & Squire, G. (2003) Quantifying landscape-scale gene flow in oilseed rape. *Final report DEFRA project* RG0216: 1-50

Rao, V. S. P. (2000) "Principles of weed science: A reference-cum-textbook," Second ed. Science Publishers Inc., Enfield. U.S.A. pp. 23-24.

Robinson, N., Armstead, S., & Bowers, M.D. (2012) Butterfly community ecology: the influences of habitat type, weather patterns, and dominant species in a temperate ecosystem. *Entomologia Experimentalis et Applicata* 145: 50-61

Salisbury, E. J. and (1961). Weeds and Aliens. Collins. London. -

Schafer, M. G., Ross, A. A., Londo, J. P., Burdick, C. A., Lee, E. H., Travers, S. E., Van de Water, P. K., & Sagers, C. L. (2011) The Establishement of Genetically Engineered Canola Populations in the U.S. PLoS ONE 6(10): e25736 , 1-4.

SCIMAC (2003). Supply Chain Initiative on Modified Agricultural Crops. www.scimac.org.uk -

Senior, I.J. & Bavage, A.D. (2003) Comparison of genetically modified and conventionally derived herbicide tolerance in oilseed rape: A case study. *Euphytica* 132: 217-226

Shelton, A. (2012) Genetically engineered vegetables expressing proteins from Bacillus thuringiensis for insect resistance: Successes, disappointments, challenges and ways to move forward. *GM Crops and Food: Biotechnology in Agriculture and the Food Chain* 3: 175-183

Silva, R. & Furlong, M.J. (2012) Diamondback moth oviposition: effects of host plant and herbivory. *Entomologia Experimentalis et Applicata* 143: 218-230

Simard, M.J., Légère, A., Pageau, D., Lajeunesse, J., & Warwick, S. (2002) The Frequency and Persistence of Volunteer Canola (*Brassica napus*) in Québec Cropping Systems. *Weed Technology* 16: 433-439

Snow, A.A., Andersen, B., & Jørgensen, R.B. (1999) Costs of transgenic herbicide resistance introgressed from Brassica napus into weedy B-rapa 82. *Molecular Ecology* 8: 605-615

Squire, G. R., Charters, Y. M., & Mackay, G. R. (1999) Distribution, Persistence and Geneflow of Oilseed Rape in the Tayside Study Area, *In Proceedings Crop Protection in Northern Britain 1999*.

Squire, G.R., Brooks, D.R., Bohan, D.A., Champion, G.T., Daniels, R.E., Haughton, A.J., Hawes, C., Heard, M.S., Hill, M.O., May, M.J., Osborne, J.L., Perry, J.N., Roy, D.B., Woiwod, I.P., & Firbank, L.G. (2003) On the rationale and interpretation of the Farm Scale Evaluations of genetically modified herbicide-tolerant crops. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences* 358: 1779-1799

Squire, G. R., Allnutt, T., Boffey, C. W. H., Cullen, D., Daniels, R. E., Ford, L., Henry, C., Jacobs, J. H., Kightley, S., Kilpatrick, J. B., Lutman, P. J., McNicoll, J. W., Osborne, J. L., Ramsay, G., Scrimgeour, C., Shearan, A., Swain, J. L., Sweet, J. B., & Young, M. W. (2008) *Factors affecting cross pollination of oilseed rape growing under UK conditions*, DEFRA and RERAD, London Final Report RG0125. -

Squire, G.R., Breckling, B., Pfeilstetter, A.D., Jorgensen, R.B., Lecomte, J., Pivard, S., Reuter, H., & Young, M.W. (2011) Status of feral oilseed rape in Europe: its minor role as a GM impurity and its potential as a reservoir of transgene persistence 6. *Environmental Science and Pollution Research* 18: 111-115

Squire, G. R., Lecomte, J., Hüsken, A., Soukup, J., & Messéan, A. (2012) "Contributions of Pollen and Seed to Impurity in Crops - A Comparison of Maize, Oilseed Rape and Beet," *In Genetically Modified and Non-Genetically Modified Food Supply Chains - Co-Existence and Traceability*, Y.Bertheau, ed., Blackwell Publishing Ltd, Chichester. pp. 23-34.

Stamatelatou, K., Turley, D., Laybourn, R., Flénet, F., Quinsac, A., Marriott, R., Antonopoulou, G., Lyberatos, G., Rouilly, A., & Vaca-Garcia, C. (2012) "Farming and Harvesting," *In Advanced Oil Crop Biorefineries*, RSC Publishing. p. 50.

The Scottish Government (2008). Agricultural Census statistics, 1982-2007. www.scotland.gov.uk -

Thirsk, J. (1997) Alternative Agriculture - A History From The Black Death To The Present Day. Oxford University Press Inc., New York. 1-376

Thompson, G.G., Withers, P.C., Pianka, E.R., & Thompson, S.A. (2003) Assessing biodiversity with species accumulation curves; inventories of small reptiles by pit-trapping in Western Australia. *Austral Ecology* 28: 361-383

US Environmental Protection Agency Washington DC (1998) Guidelines for Ecological Risk Assessment. *http://www.epa.gov/raf/publications/guidelines-ecological-risk-assessment.htm*: 1-188

Ward, J. T., Basford, W. D, Hawkins, J. H, and Holliday, J. M. (1985) Oilseed Rape. Farming Press Ltd. Ipswich. 1-298

Warwick, S.I., Simard, M.J., Legere, A., Beckie, H.J., Braun, L., Zhu, B., Mason, P., Seguin-Swartz, G., & Stewart, C.N. (2003) Hybridization between transgenic *Brassica napus L*. and its wild relatives: *Brassica rapa L*., *Raphanus raphanistrum L*., *Sinapis arvensis L*. and *Erucastrum gallicum* (Willd.) OE Schulz. *Theoretical and Applied Genetics* 107: 528-539

Warwick, S.I., Legere, A., Simard, M.J., & James, T. (2008) Do escaped transgenes persist in nature? The case of an herbicide resistance transgene in a weedy *Brassica rapa* population. *Molecular Ecology* 17: 1387-1395

Warwick, S.I., Beckie, H.J., & Hall, L.M. (2009) Gene Flow, Invasiveness, and Ecological Impact of Genetically Modified Crops
79. *Year in Evolutionary Biology 2009* 1168: 72-99

Weber, E. (2003) Invasive plant species of the world. CABI Publishing. Oxon. 1-548

Wei, J.N., Wang, L.Z., Zhu, J.W., Zang, S.F., Nandi.O.I., & Kang, L. (2007) Plants attract Parasitic Wasps to defend themselves against insect pests by releasing Hexenol. *PLoS ONE* 9: -

White, A.D., Lyon, D.J., Mallory-Smith, C., Medin, C.R., & Yenish, J.P. (2006) Feral rye (*Secale cereale*) in agricultural production systems. *Weed Technology* 20: 815-823

Wilkinson, M.J., Elliott, L.J., Allainguillaume, J., Shaw, M.W., Norris, C., Welters, R., Alexander, M., Sweet, J., & Mason, D.C. (2003) Hybridization Between *Brassica napus* and *B. rapa* on a National Scale in the United Kingdom. *Science* 302: 457-459

Witte, J.P.M., He, F., & Groen, C.L.G. (2008) Grid origin affects scaling of species across spatial scales
21. *Global Ecology and Biogeography* 17: 448-456

Zapiola, M.L. & Mallory-Smith, C.A. (2012) Crossing the divide: gene flow produces intergeneric hybrid in feral transgenic creeping bentgrass population. *Molecular Ecology* 21: 4672-4680

Zapiola, M.L., Campbell, C.K., Butler, M.D., & Mallory-Smith, C.A. (2008) Escape and establishment of transgenic glyphosate-resistant creeping bentgrass *Agrostis stolonifera* in Oregan, USA: a 4 year study. *Journal Applied Ecology* 45: 486-494